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A COMPARATIVE STUDY OF ANTIBACTERIAL ACTIVITY OF CRUDE EXTRACTS PREPARATIONS OF FIVE MEDICINAL PLANTS: *OCIMUM SANCTUM, AJADIRACHTA INDICA, PUNICA GRANATUM, PSIDIUM GUAJAVA,* AND *SYZYGIUM AROMATICUM* AGAINST MULTI DRUG RESISTANT BACTERIA OF CLINICAL ORIGIN.

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ABSTRACT

Aims: The aim of the present study was to investigate the antibacterial activity of crude extracts of Ocimum sanctum, Ajadirachta indica, Punica granatum, Psidium guajava, and Syzygium aromaticum against multi drug resistant strains isolated form community acquired infection The objective of this research was to evaluate the potential of plant extracts on standard microorganism strains as well as multidrug resistant bacteria which were isolated from hospitals. The important necessity and potentiality of medicinal plants in the practice of medicinal today is well established and cannot be overlooked. The present study is an attempt to assess the variation of drug resistance among bacterial pathogens isolated from community patients in hospitals. Hence, more studies pertaining to the use of plants as therapeutic agents should be emphasized, especially those related to the control of antibiotic resistant microbes. **Methodology**:The medicinal plants used were Ocimum sanctum, Syzygium aromaticum, Punica granatum, Azadirachta indica, Psidium guajava. All of these five species were collected from Birala hospital garden in Gwalior district MP. Plants sample were washed three times by water, dried and powdered for farther used. Clinical isolates of the following organisms: Methicillin-sensitive and resistant S. aureus, Ciprofloxacin-susceptible and resistant P.aeruginosa, and two ceftizoxime-susceptible and resistant E. aerogenes and E. coli.

All bacteria were obtained from clinical Microbiology Laboratory, Birla Hospital, and Gwalior MP. Four out of three bacteria used (pseudomonas aeruginosa, E. aerogenes, E. coli) were Gram negatives and one (S. aureus) was gram positive. There was significant variation in the antibacterial activities (DIZ value) of different extract. **Results**: In our study we observed highest antibacterial activity in the extracts of the Punica granatum (Pomegranate) showed the highest antimicrobial activity in methanol extract for Ent. aerogenes and the less effect was observed in ethanol extract on E. coli. The DIZ value (diameter of zone of inhibition) of pomegranate were between 19-26 mm. Syzygium aromaticum (clove) plant extract (flower buds) at a same concentration showed the highest antimicrobial activity in ethyl acetate extract against E. coli and the lowest antibacterial activity in ethanol extract against S. aureus. While A. indica (Neem) plant extract (leaves) observed in highest antibacterial activity in methanol extract against Pseudomonas aeruginosa and the lowest antibacterial activity in same extract on E. coli. Then Ocimum sanctum (basil) plant extract (leaves) showed the highest antimicrobial activity in ethyl acetate extract against E. aerogenes and no inhibition was seen on Ocimum sanctum (basil) methanol extract with E. coli. Psiduam guajava (guava) plant extract (leaves) showed the highest antimicrobial activity in methanol extract in ethanol against E. aerogenes and the lowest antibacterial activity in ethanol against S. aureus.

KEY WORDS: Punica granatum, drug resistance, respiratory infections, bacteria**.**

1.INTRODUCTION

The use of antibiotics in agriculture, livestock and poultry has accelerated the development of antibiotic resistant strains of microbial pathogens, potentially complicating treatment for plants, animals and human (1). The continues spread of multi drug resistant pathogens has become a serious threat to public health and a major concern for infection control practitioners worldwide (2). The problem of microbial resistance is growing, and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem, for example, to control the use of antibiotic, perform research to better understand the genetic mechanisms of resistance, and continue studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (3). Such a fact is a cause

for concern, because of the number of patients in hospitals who have suppressed immunity and due to new bacterial strains which are multiresistant.

Antibiotics are chemical substances produced from various microorganisms (bacterial and fungus) that kill or suppress the growth of other microorganisms. The term is also uses for synthetic antimicrobial agents such as sulfonamides and quinolones (4). Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. The wide use of antibiotics in the treatment of bacterial infections has led to the emergence and spread of resistant strains. The emergence of multiple drug resistant bacteria (MDR) has become a major cause of failure of the treatment of infectious disease (5). As a result, society is facing one of the most serious public health dilemmas over the emergence of infectious bacteria displaying resistance to many and in some cases, effective antibiotic (6).

It has been reported that between the years 1983 and 1994 (7), the systematic screening of antibacterial plant extracts represents a continuous effort to find new compounds with the potential to act against multiresistant bacteria. According to World Health Organization (8), medicinal plants would be the best source to obtain a variety of drugs. For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade with more intensive studies for natural therapies.

The aim of the present study was to investigate the antibacterial activity of crude extracts of Ocimum sanctum, Ajadirachta indica, Punica granatum, Psidium guajava, and Syzygium aromaticum against multi drug resistant strains isolated form community acquired infection Hence, more studies pertaining to the use of plants as therapeutic agents should be emphasized, especially those related to the control of antibiotic resistant microbes. The objective of this research was to evaluate the potential of plant extracts on standard microorganism strains as well as multidrug resistant bacteria which were isolated from hospitals. The important necessity and potentiality of medicinal plants in the practice of medicinal today is well established and cannot be overlooked.

2. MATERIALS AND METHODS

The medicinal plants used were Ocimum sanctum, Syzygium aromaticum, Punica granatum, Azadirachta indica, Psidium guajava. All of these five species were collected from Birala hospital garden in Gwalior district MP. Plants sample were washed three times by water,

dried and powdered for farther used. Clinical isolates of the following organisms: Methicillin-sensitive and resistant S. aureus, Ciprofloxacin-susceptible and resistant P.aeruginosa, and two ceftizoxime-susceptible and resistant E. aerogenes and E. coli. All bacteria were obtained from clinical Microbiology Laboratory, Birla Hospital, and Gwalior MP.The clinical isolates of bacteria were inoculated on nutrient agar slopes and incubated overnight at 37' C. these cultures were stored in a refrigerator at 4'C. Fresh slope cultures were prepared every 3-4 weeks. Nutrient broth was used as the basic liquid culture medium for growing the overnight cultures.

Plant Parts Used for Extracts Preparation

The following plant parts were used for extractions.

Basil (OcimumSanctum) - leaves

Clove (Syzygium aromaticum) -dried buds

Pomegranate (Punica granatum) - pericarp

Neem (Azadichta indica) - leaves

Guava (Psidium guajava) - leaves

Medicinal Plant Extraction

The leaves of the plants were air dried at room temperature for 3 weeks and grounded to coarse powder 5.0 g. of the powder was placed in 25 ml. of ethanol methanol and ethyl acetate in conical flask and kept in rotary shakes at 150 pm for 24 hours. After 24 hours, it was filtered and the solved evaporated.Theextractswerestored in sample bottles at $4^\circ C$ prior to use.Methanol ethanol and ethyl acetate Solvents which were used as the test extracts for antimicrobial activity assay.

Samples of plant extracts used for the study

Antibacterial Sensitivity Testing

Most of the antibiotics used by the previous researchers used agar diffusion assay to determine the antibacterial activity of extracts, this technique work well with defined inhibitors but when examining extracts containing unknown components there are problems leading to the false positive and false negative results. The antimicrobial effect may be inhibited or increased by extrinsic factors or contaminants. The most widely used alternative technique is the dilution method. Four out of three bacteria used (pseudomonas aeruginosa, Ent. aerogenes, E. coli) were Gram negatives and one (S. aureus) was gram positive. There was significant variation in the antibacterial activities (DIZ value) of different extract.

The MHA agar was punched with 6mm diameter wells. The inoculums were spread on to the agar plates using sterile swabs and then the wells were filled with extracts. To evaluate the efficiency of the methodology, each extract was inserted simultaneously in a hole made (50µl) in new plates. The plates were then incubated at 37ºC for 24 hours. After incubation, zone of growth inhibition for each extract was measured in millimeters using veneer calipers. Each extract was tested three times.

3. RESULTS AND DISCUSSION

The effect of fixed volumes of O. sanctum, A. indica, Punica granatum, Psidium guajava and Syzygium aromaticum on S. aureus, E. aerogenes ,E. coli, Pseudomonas aeruginosa is tabulated in tables 1-4 and the graphic representation of the same results are presented in graphs. We observed highest antibacterial activity in the extracts of the Punica granatum (Pomegranate) .All our extracts showed the highest antimicrobial activity in methanol extract for E. aerogenes and the less effect were observed in ethanol extract on E. coli. The DIZ value (diameter of zone of inhibition) of pomegranate were between 19-26 mm. Syzygium aromaticum (clove) plant extract (flower buds) at a same concentration showed the highest antimicrobial activity in ethyl acetate extract against E. coli and the lowest antibacterial activity in ethanol extract against S. aureus. While A. indica (Neem) plant extract (leaves) observed in highest antibacterial activity in methanol extract against Pseudomonas aeruginosa and the lowest antibacterial activity in same extract on E. coli. Then Ocimum sanctum (basil) plant extract (leaves) showed the highest antimicrobial activity in ethyl acetate extract against E. aerogenes and no inhibition was seen on Ocimum sanctum (basil) methanol extract with E. coli. Psidium guajava (guava) plant extract (leaves) showed the highest antimicrobial activity in methanol extract in ethanol against E. aerogenes and the lowest antibacterial activity in ethanol against S. aureus.

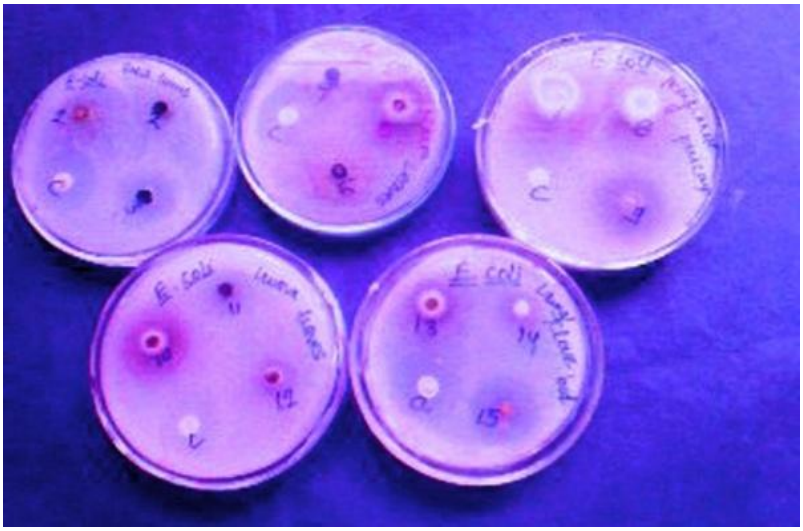
Antimicrobial drug resistance in human bacterial pathogens is a continuing worldwide issue and as a consequence, effective treatment and control of such organisms remains an important challenge. Bacterial resistance has appeared for every major class of antibiotic (9). Since their introduction the emergence of resistance to antibiotics has become increasingly evident, particularly for important pathogens such as Escherichia coli (E. coli), Salmonella spp., Campylobacter spp., Enterococcus spp. and Staphylococcus spp. Over the last decade research into the antimicrobial properties of traditional plant based medicines has been revisited (10). Numerous plants have been screened for antimicrobial properties, for example Holetz and colleagues (11) tested 13 plants used in Brazilian traditional medicine and they demonstrated activity against bacteria such as Staphylococcus aureus (S. aureus) and E. coli. (14) tested 172 plant species used in Puerto Rico and they demonstrated that 14 of these showed activity against bacteria including S. aureus and E. coli.

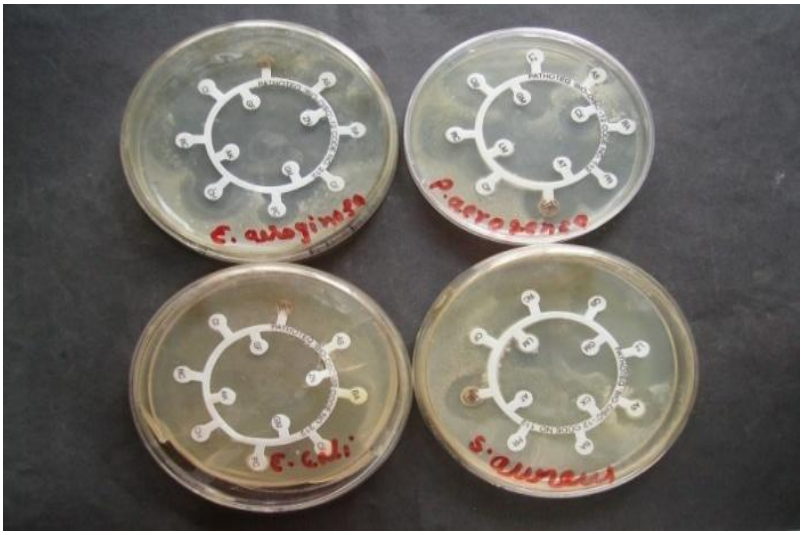
Punica granatum L. (Punicaceae) referred to in English as pomegranates, have been high lighted in many studies as having antimicrobial activity against a range of both Gram positive and negative bacteria (15,16). Prashanth and colleagues tested a number of extracts of pomegranates against a range of bacteria (S. aureus, E. coli, Klebsiella pneumoniae, Proteus vulgaris, Bacillus subtilis (B. subtilis) and Salmonella typhi), and they found activity against all isolates. Braga and colleagues observed that pomegranate extracts were able to inhibit not only the growth of S. aureus but also the production of enterotoxin. The methanolic extract derived from 200 g of dried pomegranate produced bactericidal effects at 1% (v/v) over an extended incubation period (50 hours), demonstrating longevity of action.

Many previous researches reported the antibacterial activity of O. sanctum, A, indica, P. granatum, Syzygium aromaticum, Psidium guajava. Different plant part from it displayed good antimicrobial activity against S. aureus, P. aeruginosa and E. coli at various concentrations (17, 18). The methanolic extract from aerial part and leaf at 20 mg/ml (13) as well as from leaves at 2 mg/ml (19) does not inhibit Gram negative bacteria, E. coli and P. aeruginosa. But they showed activities against Gram positive bacteria, S. aureus. The 80% ethanolic extract (20) and 88% ethanolic extract (21) do not inhibit growth of S. aureus, P. aeruginosa and E. coli (22) and 88% ethanolic extract (23,24). Aqueous extracts did not have activity against P. aeruginosa, E. coli either (25). The previous studies in O. sanctum reported that methanol, ethanolic, and ethyl acetate extract inhibited growth of S. aureus and E. coli (26), while 88% ethanolic extract of leaves and flowers had no activity against S. aureus (24).

Essential oil of O. basilicum showed antibacterial activity against S. aureus and E. coli (27). An aqueous extract inhibited E. coli but not S. aureus (28). Methanolic extract of whole plant at 10 mg/ml inhibited growth of S. aureus and 40 mg/ml for E. coli and P. aeruginosa (29). Variation between the results reported by previous workers and our studies could be due to differences in the plants physiological state of development, diurnal and seasonal variation, environmental condition, part of the plants, extraction procedure, and concentration of the crude extracts and strains of test microorganism (30). Our study states that the plant extracts were found to have antibacterial activity against drug-susceptible and resistant bacteria. This has clearly indicated that antibiotic resistance does not interfere with the antimicrobial action of plant extracts and these extracts might have different mode of action on test organisms (31).Among All these extracts P. granatum, were highly active against clinical isolates,

Both Gram Positive And Gram Negative Bacteria





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Antibiogram Of Test Microbial Samples

Table No1- Effect of Plants extracts in different solvent on S.aureus

Bacteria	**Plant extract**	**Zone of inhibition in mm.**				
	Name of solvent	*O.Sanctum*	*A. Indica*	*P. granatum Aromaticum*	*P. Gaujava*	*Syzygium*
S.aureus	Methanol	20	15	24	14	15
	Ethanol	12	14	22	10	14
	Ethyl acetate	14	12	20	13	20
	Control	22	22	22	22	23

Table No 2-Effect of Plants extracts in different solvent on E. coli.

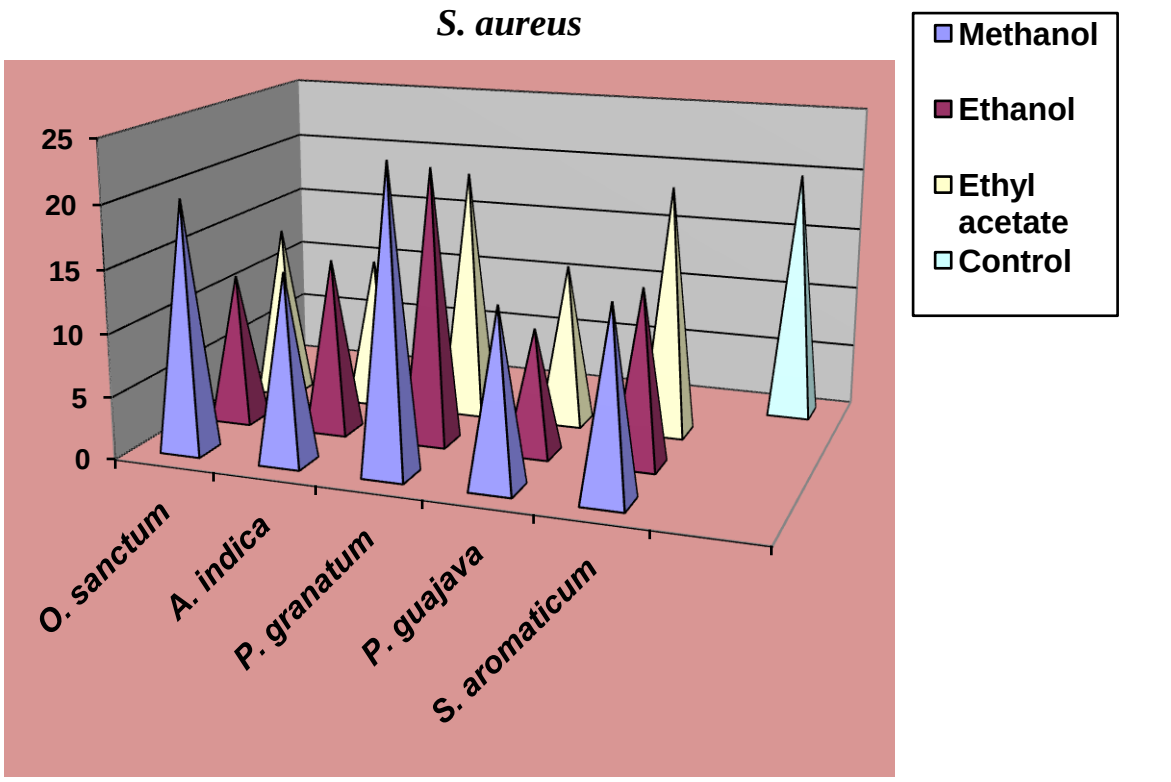
Bacteria	Plant extract	Zone of inhibition in mm.				
	Name of solvent	*O. sanctum*	*A. indica*	*P. granatum aromaticum*	*P. gaujava*	*Syzygium*
E.coli	Methanol	R	11	23	12	16
	Ethanol	10	14	19	15	18
	Ethyl acetate	14	14	22	13	25
	Control	22	22	22	22	23

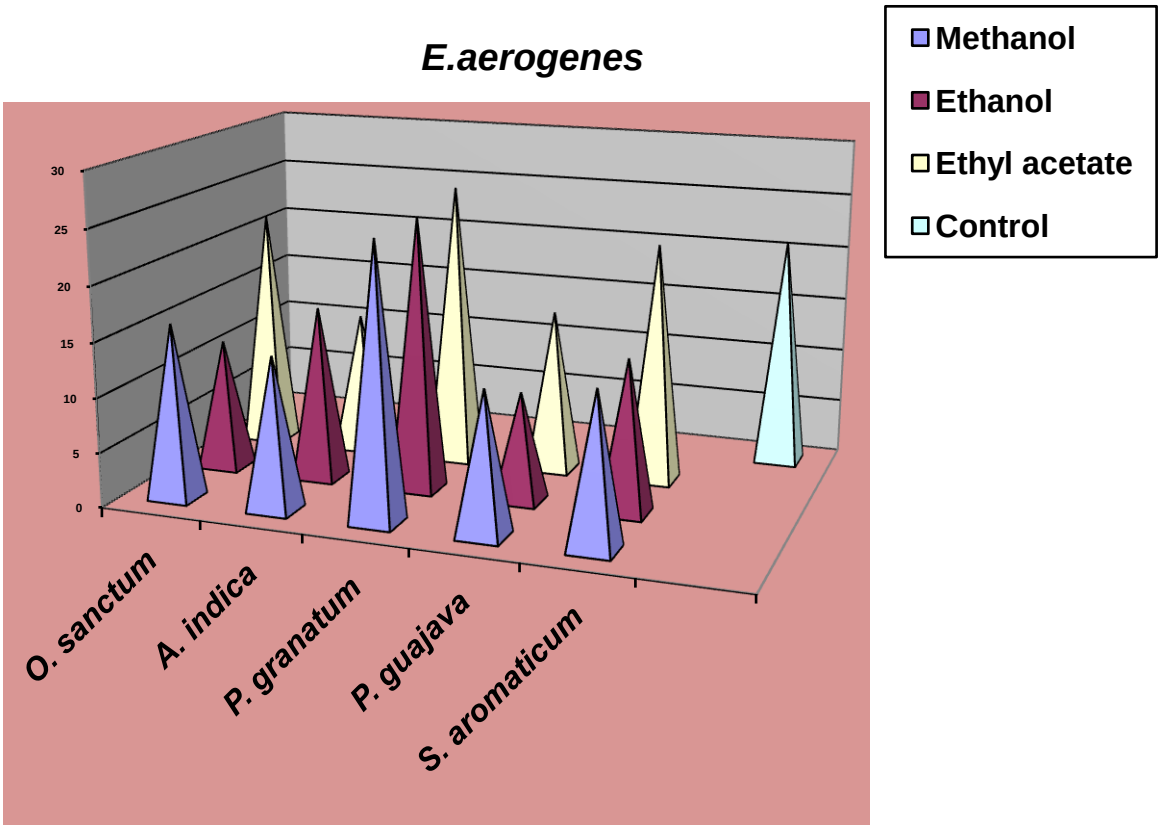
Table No3- Effect of Plants extracts in different solvent on Pseudomonas aeruginosa.

Bacteria	Plant extract	Zone of inhibition in mm.				
	Name of solvent	*O. sanctum*	*A. indica*	*P. granatum aromaticum*	*P. gaujava*	*Syzygium*
Pseudomonas aeruginosa.	Methanol	12	20	24	14	17
	Ethanol	11	19	22	17	15
	Ethyl acetate	20	15	20	13	22
	Control	30	30	30	30	30
		12	20	24	14	17

TABLE NO4-Effect of Plants extracts in different solvent on E. aerogenes

Bacteria	Plant extract	Zone of inhibition in mm.				
	Name of solvent	*O. sanctum*	*A. indica*	*P. granatum aromaticum*	*P. gaujava*	*Syzygium*
E.aerogens	Methanol	16	14	25	13	14
	Ethanol	12	16	25	10	14
	Ethyl acetate	22	13	26	15	22
	Control	21	20	21	21	21


S. aureus
Methanol
Ethanol
Ethyl acetate
Control
25
20
15
10
5
0
O. sanctum
A. indica
P. granatum
P. guajava
S. aromaticum


E.aerogenes
Methanol
Ethanol
Ethyl acetate
Control
30
25
20
15
10
5
0
O. sanctum
A. indica
P. granatum
P. guajava
S. aromaticum

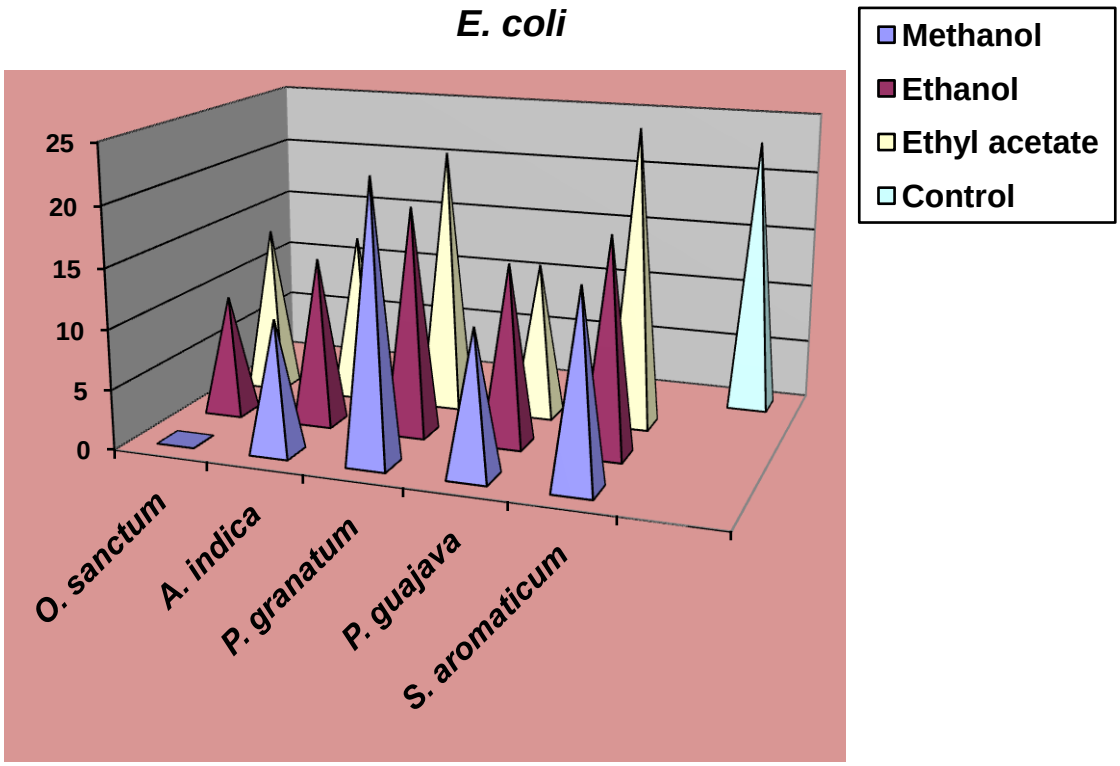


TABLE NO5-Antibiogram of clinical isolates

S.No.	Antibiotics used	Name of the organisms			Name of the organisms	
		Gram -ve			Gram +ve	
	Short code	*Enterobacter aerogenes*	*E. coli*	*Pseudomonas aeruginosa*	*Short code*	*S. aureus*
1	AS-	S	R	S	AS-	S
2	BA-	S	R	S	BA-	R
3	CF-	R	R	S	PR-	S
4	PC-	S	R	S	TE-	S
5	CH-	S	R	R	CF-	R
6	RC-	S	R	S	RC-	S
7	CI-	S	R	S	QB-	S
8	TE-	R	R	S	LS-	S
9	ZN-	S	R	S	GM-	S
10	GM-	S	R	S	CX-	R
11	AK-	S	R	S	AT-	R
12	GF-	S	R	S	LM-	S

Competing Interests

Authors have declared that no competing interests exist.

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