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THERAPEUTICALLY AND SYNERGISM EFFECT BETWEEN *ANASTATICA HIEROCHUNTICA* AND ANTIBIOTICS AGAINST MULTIDRUG RESISTANCE BACTERIA ISOLATE FROM ENDOMETRITIS

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ABSTRACT : To find out common organisms causing Endometritis, Detection sensitivity and resistance bacteria to antibiogram pattern, Determine the antibacterial & synergismeffect of *Anastatica hierochuntica* extract and antibiotics against test bacteria. In this study, 90 endocervical swabs were taken from women with suffering from endometritis attending to Al-Zahraa hospital for Maternityand Children in Al-Najaf province during the periodfrom December,2017 to February, 2018. All specimens were direct examination and cultured on different media. in addition to Evaluation of the Antibacterial bioassay of *Anastatica hierochuntica* and antibiotics against test bacteria. Out of 90 women admitted in present study, It was observed that *Neisseria gonorrhea* isolates were recorded the highest % of total isolates (32%), followed by *Staphylococcus* sp (26%), *E. coli* (12%) and *Staphylococcus aureus* (10%), in addition *Klebsiella pneumonia, Proteus* sp *Shigella* sp recorded (5%, 3%, 2%) respectively. In addition to detection Bacterial growth at various time points, this result show in methanolic extract of *Anastatica hierochuntica*to batter than aqueous extract during monitoring turbidity growth of bacteria. And investigate antibiogram sensitivity test bacteria, the result revealed in all gram positive and gram negative bacteria to resistance of all antibiogramexcept Chloramphenicol to sensitive of both bacteria.Present study shows the antibacterial and synergism effect to both aqueous and methanolic extract against test bacteria to high effect in comparation antibiotic alone. It could be finally concluded that the very small amount of the Plant extracts of *Anastatica hierochuntica* have great potential as antimicrobial compounds against pathogenic bacteria. Thus, they can be used in the treatment of endometritis caused by resistant microbes to antibiogram agent, The synergism effect from the association of chloramphenicol antibiotic with plant extracts against resistant bacteria leads to new choices for the treatment of infectious diseases. This effect enables the use of the respective antibiotic when it is no longer effective by itself during therapeutic treatment.

Key words : Endometritis, *Anastatica hierochuntica*, antibiotics, antibacterial, synergism.

INTRODUCTION

Anastatica hierochuntica, a member of the family: Brassicaceae is commonly called 'Kaff-e-Maryam', is a well known wasteland region medicinal plant. Novel melanogenisis inhibitor flavonoids with antioxidant probable were isolated from it (Nakashima *et al*, 2010). is commonly called "Kaff maryam" or "Rose of Jericho", which is a small grey winter yearly herb, found in the arid regions of Saudi Arabia, Egypt, North Africa, Jordan,, Iraq and Kuwait (El-Ghazali *et al,* 2010). These plantare efficient in the treatment of infectious diseases because consider source antimicrobials as simultaneously mitigating many of the side effects that are often linked with synthetic antimicrobials (Parekh *et al*, 2005). The species of *Anastatica hierochuntica* from crucifiers' are an important dicotyledonous plant family, is well-known for its therapeutic properties as a hepato-protective plant, hypoglycemic and diuretic. It is used in traditional medicine for uterine bleeding and to facilitate the expulsion of dead fetus, to treat gastrointestinal disorders, depression, high blood pressure, indigestion, headache, fever, malaria, epilepsy, heart disease and infertility (Hegazy and Kabiel, 2007). However, many these studies had known and quantified a number of minerals and phenolic compounds from this plant used for treating health disorders. other than fewer studies were available on the histological effect of the prolonged use of *Anastatica hierochuntica* on different body organs (Ihsanullah, 2012). For the treatment of microbial infections, antimicrobials of plant origin are considered as effective due to less side effects (Parekh *et al*, 2005).

Endometriosis is a common estrogen-dependent disorder that can result in substantial morbidity, as well as pelvic pain, multiple operations, and infertility. recent treatment of endometriosis is mainly based on surgery and ovarian suppressive agents. Approximately only half of women with endometriosis get pain relief from existing medical or surgical treatments. Currently available medical therapies are designed to suppress estrogen synthesis, inducing atrophy of ectopic endometriosis implants or interrupting the cycle of stimulation and bleeding. Oral contraceptive, androgenic agents, progestin'sand gonadotropin-releasing hormone analogues have all been successfully used in the treatment of endometriosis (Ferrero *et al*, 2005). Clinical endometritis is define as the presence of a purulent discharge detectable in the vagina 21 days or more post partum, or mucopurulent discharge detectable in the vagina after 26 days post partum. The occurrence of clinical endometritis is around 10 to 20%, with variation between breed, country and herd; a typical study reported that 16.9% of 1,865 cows were affected in Canada (LeBlanc *et al*, 2002).

The incidence of bacteria in the uterus causes inflammation, histological lesion of the endometrium and delays uterine involution (Sheldon *et al*, 2003). Antibiotics are one component frequently used in the vancomycin treatment of clinical endometritis. For clinicians there is a require of rapid microbiological diagnosis so that adequate treatment of the infection can be performed (Kask *et al*, 1998).

Aims and objective

1. To find out common organisms causing Endometritis.
2. Detection sensitivity and resistance bacteria to antibiogram pattern.
3. Determine the antibacterial & synergism effect of *Anastatica hierochuntica* extract and antibiotics against test bacteria.

MATERIALS AND METHODS

Plant material

Anastatica hierochuntica was brought from local market of Najaf city. The plant was separatedinto seedfrom stems, after that the seed werewashed with tap water and dried at room temperature (25°C). The dried seed material wasground to fine powder.

Extraction of plant materials

❖ **Methanolic extract**

The plant extracts were prepared using themodified method of Matkowski and Piotrowska (2006). Briefly, 10g of the dried powder from the plant were flooded separately in 100ml of methanol (100%). Then, each mixture was refluxed in awater bath in the dark at 45°C. The extracts were filtered through Whatman filter paper No. 42. The collected filtrates were dried under vacuum at 40°C by using a rotary evaporator or oven the extraction is repeat twice. The resulting residue was re-dissolved in methanol.

❖ **Aqueous extract**

Powder sample of 20g is dissolve in 200ml of distilled water in conical flask the mouth of the conical flask was enclosed with aluminum foil and incubated in slow heat for 2 h. The residue was filtered by using muslin cloth followed by Whatman No.1 filter paper, then centrifuged at 6000 rpm for 10 min. The supernatant was collected and further boiled till the volume was reduced to one-fourth of the original volume of the water used and finally is evaporate by rotary evaporator or oven and stored at 4°C (Al Sobeai, 2016).

❖ **Concentration of plants extracts**

Stock solution was prepared for each extract by dissolving 1000mg of dried extract with 1ml of distilled water for aqueous extract and alcohol (methanol) extract, so the final concentration of extract would be 1000 mg/ml, which was used against test bacteria (Nwachukwu and Uzoeto, 2010).

Specimen collection and culture

In this study, 90 endocervical swabs were taken from women with suffering from endometritis attending to Al-Zahraa Hospital for Maternityand Children in Al-Najaf province during the period from December, 2017 to February, 2018. All specimens were cultured on the Nutrient agar, MacConkey agar, Mannitol salt agar, Blood agar, SS-agar, Chocolate agar, plates and incubated at 37°C under aerobic condition for 18 - 24 hour.

Evaluation of the antibacterial bioassay

1. Monitoring bacterial growth at various time point

To study the bacterial growth at various time point from *Anastatica hierochuntica* extract, this method is used by Baeshin *et al* (2005) with some modification was following :

- ✓ Single colonies of the tested bacteria were used for inoculation of nutrient broth and were incubated at 37°C for 24 h.
- ✓ methanolic & aqueous extract was prepared at concentration (100)mg/ml.
- ✓ intake 1ml from test bacteria at a concentration of 1.5×10^8 cells/ml with 1ml methanolic & aqueous extract and incubated at 37°C for

(1,6,18,24,48) hours.

- ✓ The antibacterial activities of the *A. hierochuntica* was evaluated by spectrophotometer to measuring the Turbidity Growth of bacterial at a concentration (100) mg/ml at optical density 600 nm.

2. Antibiogram sensitivity test

The bacteria isolates were tested for their sensitivity to a variety of chemotherapeutic agents by use disc diffusion method. The test was performed using Mueller Hinton agar by employing (12) antibiogram discs. Now, the zones of growth inhibition around each of the antibiotic discs are measured to the nearest millimeter. The diameter of the zone is related to the susceptibility of theisolate and to the diffusion rate of the drug through the agar medium (CLSI, 2016).

3. Antibacterial and synergism effect

The isolates at a concentration of 1.5×10^8 cells/ml (adjusted to the 0.5 Mc Farland turbidity standards) were inoculated onto thesurface of Mueller Hinton agar plates. Cut media into three wells (6mm diameter) by cork borer and put 10 µl in each wells of both extract (methanolic & aqueous). Subsequently, the synergism effect, the antibiotic disks of 6 mm indiameter saturated with 10 µl plant extractwere placed on the surface of each inoculatedplate. The plates were incubated for 24 h at37°C. The diameters of clear zones weremeasured and compared with that of theantibiotic alone (Betoni *et al*, 2006).

Statical analysis

Data were analyzed for effects of Antibacterial and synergism effect of aqueous & methanolic extracts of against testbacteria *Anastatica hierochuntica*. The level of significance was tested at $P < 0.05$. A statistical program Statistical Package for the Social Sciences (SPSS) version 19 was used to perform the statistical analysis.

RESULTS AND DISCUSSION

Isolation and identification of bacteria

Various species of bacteria isolated from female with suffering from Endometritis, these sample to diagnosed by direct examination and culture. Table 1 shows the distribution of bacteria isolate from Endometritis in (90) patients.

It was observed that *Neisseria gonorrhea* isolates were recorded the highest % of total isolates (32%), this result was in agreement with a study carried at the Najaf city (Zainab, 2006) followed by *Staphylococcus* sp (26%) *E.coli* (12%) and *Staphylococcus aureus* (10%), in addition *Klebsiella pneumonia, Proteus* sp, *Shigella* sp recorded (5%, 3%, 2%), respectively. In cases of Endometritis, our study did not constituent with Takamtha *et al* (2013) by findings who isolated *E. coli* (24.07%), *Staphylococcus* spp. (11%), *Bacillus* spp. (20.37%) and *Streptococcus* spp. (14.81%). According to Williams *et al* (2005) the bacteria isolated belonged to obligate uterine pathogens (*A. pyogenes, E. coli*), potential uterine pathogens (non hemolytic B Streptococci) and opportunistic bacteria (*Klebsiella* spp*, Proteus* spp. and coagulase-negative staphylococci).

Table 1 : Distribution of bacteria isolate from Endometritis.

Type of bacteria		Number	Percentage
Gram positive	*S. aureus*	10	11
	Staphylococcus sp	26	28.8
Gram negative	*N.gonorrhea*	32	35.5
	E. coli	12	13
	K. pneumoniae	5	5.5
	Shigella sp	2	2
	Proteus sp	3	3
Total		**90**	**100%**

Bacterial growth at various time points

Antibacterial activity of aqueous and methanol extract from *Anastatica hierochuntica* against the seven tested bacteria was further explored by incubating them in medium containing at concentrations of both extract of *A. hierochuntica* and monitoring the bacterial growth. Under the concentration treatment (100mg/ml) of both extract, the bacterial growth turbidity decrease continuously during the whole period of treatment (1h-48h).

The result monitoring the bacterial growth with use aqueous extract of *A. hierochuntica* to show that the *S.aureus*, *K. pneumonia* and *Proteus* sp the turbidity growth zero at 48h, while other bacteria include *N.gonorrhea*, *Staphylococcus* sp(0.2)*, E. coli* and *Shigella* sp (0.3) at the same time (Fig. 1).

However, the methanolic extract of *A. hierochuntica* the turbidity growth show that the *S.aureus, Staphylococcus* sp*, E.coli*, *K. pneumonia* and *Shigella* sp zero at 48h, while another bacteria include *N. gonorrhea*, *Proteus* sp the turbidity growth (0.1) at 48h as show in Fig. 2.

This suggests that some of the active compounds in the methanolic extract exist in higher percent than those in aqueous extracts. Correspondingly to the finding of the present study, many studies have been reported for *A. hierochuntica* methanol and aqueous extracts with Gram

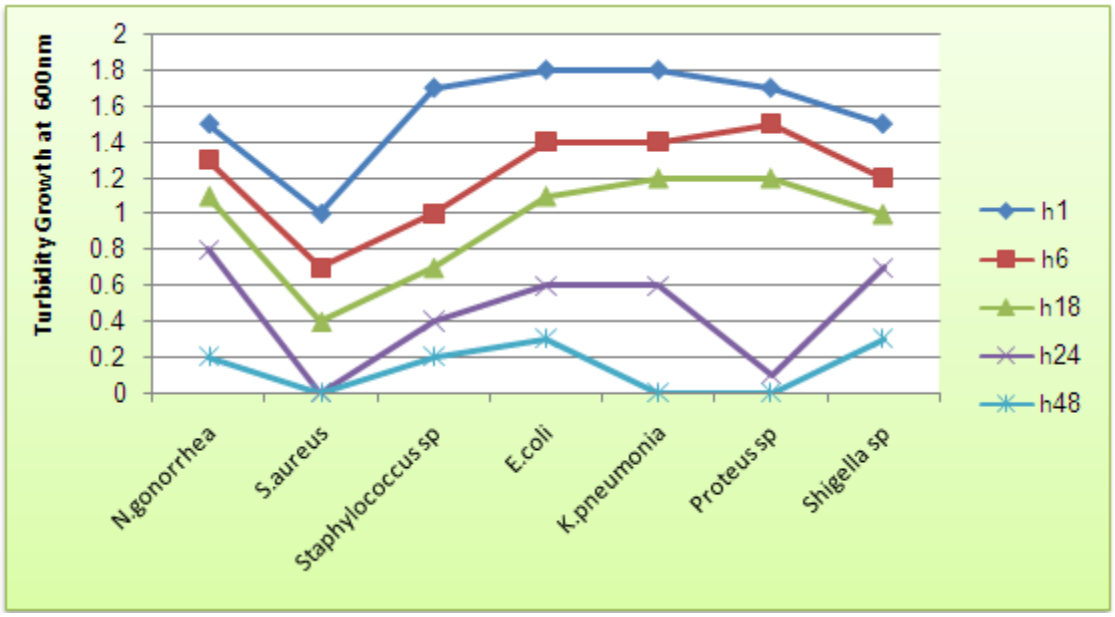

Fig. 1 : Turbidity growth of aqueous extract of *A. hierochuntica* against the tested bacteria treated with low concentration (100mg/ml) of aqueous extract.

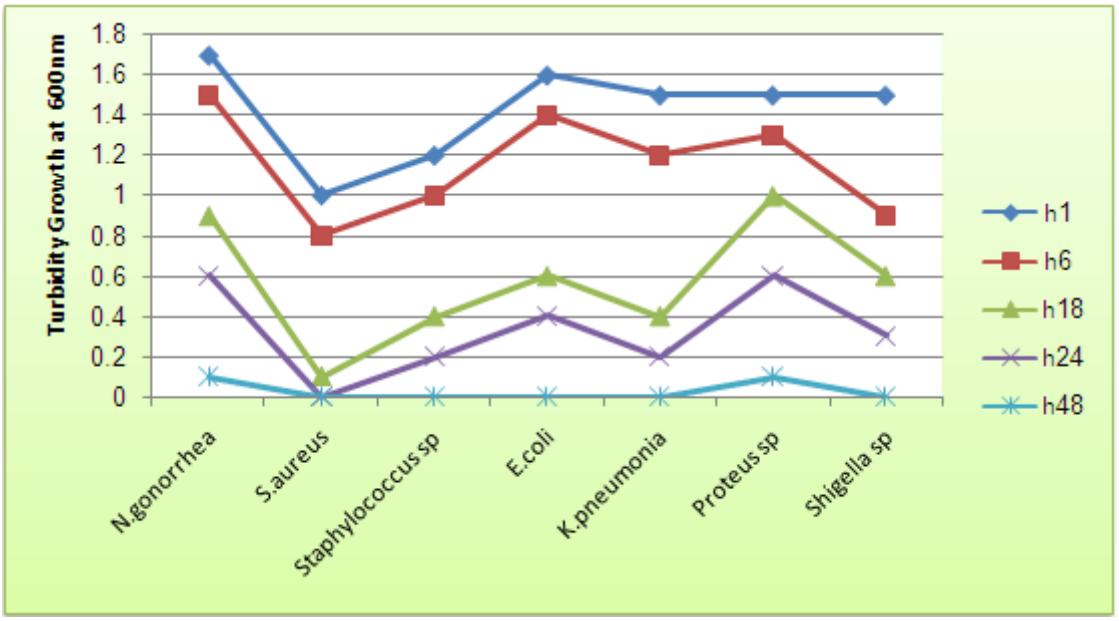

Fig. 2 : Turbidity growth of methanol extract of *A. hierochuntica* against the tested bacteria treated with low concentration (100mg/ml) of methanol extract.

negative and positive bacteria, which suggest that the plant extract may possess broad spectrum of antibiotic compounds or simply general metabolic toxin (Mohamed *et al*, 2010). The results obtained in this study clearly consistent with Al Sobeai (2016) revealed that *A. hierochuntica* possess potential inhibition effects against pathogenic bacterium, after treatment with low concentration (0.3%) of methanol extract was found to have shown the strong inhibition and broadest spectrum antibacterial activity during monitoring Turbidity Growth with use spectrophotometer against Gram positive and Gram negative bacteria. Several studies have highlighted the high sensitivity of Gram-positive bacteria compared to Gram-negative bacteria (Falleh *et al,* 2008). Current findings are also in full agreement with earlier pharmacological experiments on glucosinolates and their derived isothiocyanate present in *A. hierochuntica*. Such compounds also demonstrated profound anti-proliferative activity (Hecht, 2000).

Antibiogram sensitivity test

The susceptibility of all bacteria isolates from Endometritis was investigated using the Antibiogram sensitivity test based on the methods given in (CLSI, 2016). From all the 12 Antibiogram agents used for gram

positive bacteria include Azithromycin, Chloramphenicol, Clarithromycin were sensitive *S.aureus* while *S.epidermidis* were sensitive Chloramphenicol only, whereas, Meropenem, Ciprofloxacin were intermediate to both bacteria, while other Antibiogram were resistance has been shown in Table 2.

While gram negative bacteria to sensitive Chloramphenicol, whereas *E.coli* to sensitive and intermediate (Meropenem & Ciprofloxacin), respectively. While all gram negative bacteria to resistance another Antibiogram as shown in Table 3.

However, the efficacy of such therapeutic agents needs to be evaluated occasionally due to continuous emergence of drug resistant bacterial strains. The present study revealed that the bacterial isolates from infected uterine are significantly sensitive to nitrofurantoin, kanamycin and chloramphenicol (Tibary and Anouassi, 2001).

The antibiotic resistance results are in agreement with our works series (Benyagoub *et al*, 2013). Performance of β-lactam antibiotics and cephalosporins declines due to presence of organisms producing inactivating (β-lactamase) enzymes in postpartum uterus (Deori and Phookan, 2015). About the emergence of antibiotic resistance of microorganisms responsible of urinary tract infections (UTI) in *Bechar* (Algeria) where a total of 145 strains were isolated and have experienced an antibiogram test. Antibiotic resistance is relatively high for specific molecules, in particular beta-lactams (penicillin, oxacillin, ampicillin and amoxicillin-clavulanic acid), sulphamides (cotrimoxazole) and macrolides (erythromicin) for *E. coli* and *S. aureus*. This result consistent with other scientific papers, *E. coli* strains from the uterus were the most sensitive to chloramphenicol, enrofloxacin, gentamycin and polymixin B (Noakes, 1989). These results were not agreement with some other observations where percent sensitivity to enrofloxacin and ciprofloxacin has been reported as 92 and 96%, respectively (Mshelia *et al*, 2014). In our study, Levofloxacin was the most sensitive and penicillin was the most resistant for both gram-positive and gram-negative pathogens. The treatment of bacterial infections is increasingly complicated by the ability of bacteria to develop resistance to antimicrobial agents. Generally, indiscriminate use of antibiotics leads to development of resistant strains due to bacterial mutation (Takamtha *et al*, 2013). However, Rao and Seshagiri (1997) reported gram positive bacteria with a maximum sensitivity with Chloramphenicol (87.50%), while non lactose fermenter bacteria with least sensitivity to penicillin (3.13%).

The treatment of bacterial infections is increasingly complicated by the ability of bacteria to develop resistance to antimicrobial agents (Tenover, 2006). The more antibiotics used improperly, the more chances for bacterial mutation.

Evaluation of the antibacterial and synergism effect

The results of growth inhibitory effect in both antibacterial and synergism effect of the two plant extracts (methanolic & aqueous) and their synergism with the choose Chloramphenicol antibiotics according to the sensitivity of all test bacteria to reveal a significant less (P≥0.05), Our results showed thatthe aqueous extracts of *Anastatica hierochuntica* exhibited growth inhibitory effect against test bacteria ranged from (20-23) mm, compare Chloramphenicol with (18-20)mm, while synergism effect range from (25-30) were presented in Fig. 3.

However, the result in Fig. 4 that showed a significant (P≥0.05) to the inhibitory effect of methanolic extracts of *Anastatica hierochuntica* exhibit growth against test bacteria ranged from (25-28) mm, while Chloramphenicol

Table 2 : Antibiogram for Gram positive bacteria.

Antibiotic	*S. aureus*		*Staphylococcus* sp	
	Inhibition Zone (mm)	Antimicrobial susceptibility	Inhibition Zone (cm)	Antimicrobial susceptibility
Penicillin	0	R	0	R
Ampicillin	0	R	0	R
Oxacillin	10	R	10	R
Azithromycin	18	S	10	R
Meropenem	20	I	20	I
Tobramycin	10	R	12	R
Gentamicin	0	R	0	R
Tetracyclin	15	R	13	R
Ciprofloxacin	20	I	20	I
Levofloxacin	0	R	0	R
Chloramphenicol	20	S	18	S
Clarithromycin	18	S	10	R

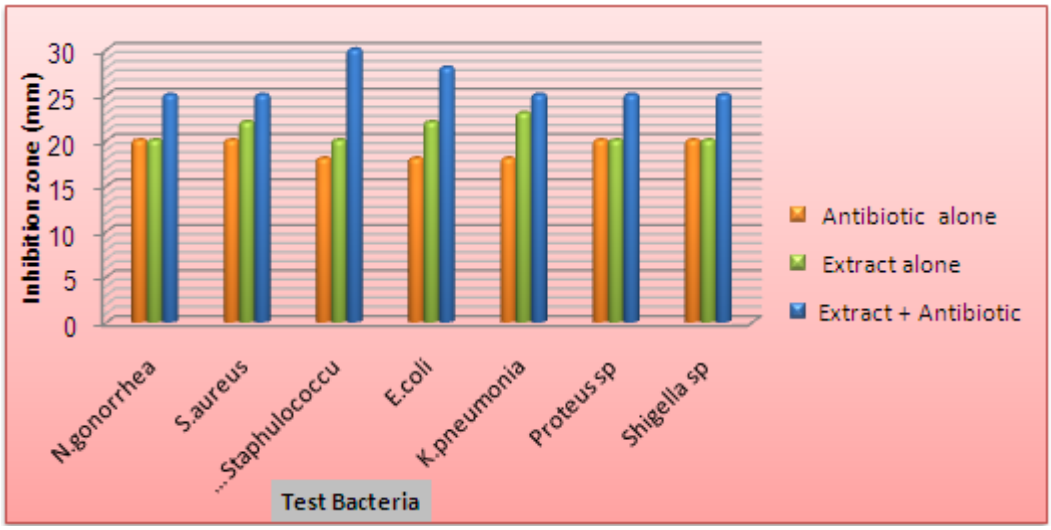

Fig. 3 : Antibacterial and synergism effect of aqueous extracts against test bacteria (all values in mm).

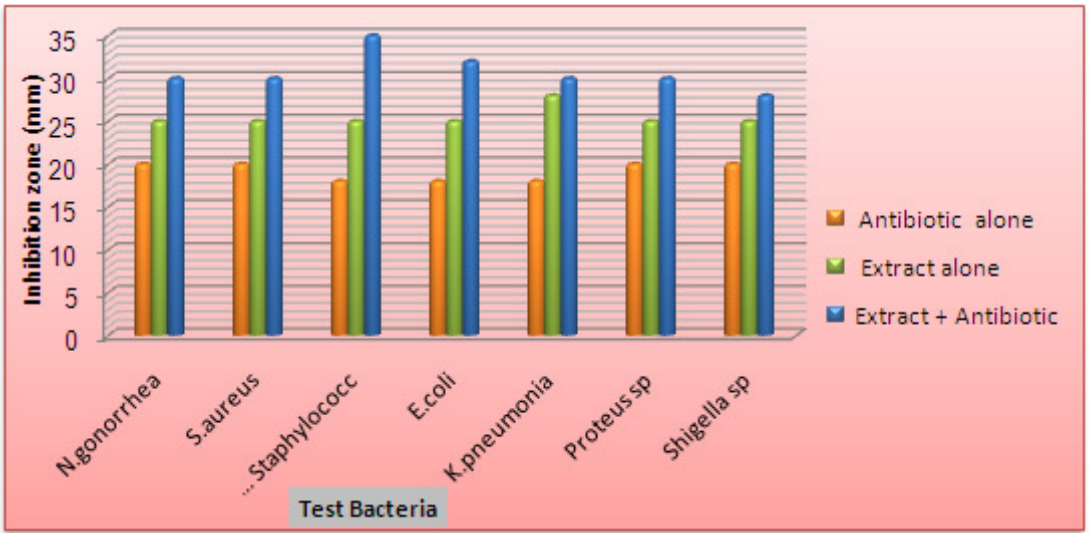

Fig. 4 : Antibacterial and synergism effect of methanolic extracts against test bacteria (all values in mm).

range from (18-20mm), whereas synergism effect range from 28-35 as shown in Fig. 4.

The mean zone of inhibition produced by the methanol and aqueous extracts from *A. hierochuntica* against tested bacterial species were greatly than to those produced by commercial standard antibiotic disc. The difference in inhibitory activities of different extract may be due the solubility of phytochemicals in different solvents. There are many reports on solubility of different bioactive components in different solvents according to their polarity (Marjorie, 2001).

The potency of *Anastatica hierochuntica* leaves as antibacterial is attributed to the action of phytochemical compounds is contains, which are secreted by plant naturally, in response to environmental pressure or as a defense mechanism to animal attacks or plant diseases, these compounds normally extracted in polar extracts included alkaloids, flavonoids and some phenols, ornon-polar compounds may include terpenes andsterols. Antibacterial activity may involve complex mechanisms, like the inhibition of the synthesis of cell walls and cell membranes and proteins (Oyaizu *et al*, 2003). This results help to explain the therapeutic effect ofthe plant. The antibacterial activities against both gram positive and gram negative bacteria may indicate the presence of broad spectra. In our study, the plant extracts had different antibiotic compounds or simply metabolic toxins in plant extracts (Moniharapon and Hashinaga, 2004). This may be described by the fact that the secondary metabolites responsible for demonstrating antibacterial activity are greatly dependent on solvent system and collection process of metabolites from the plant sources (Rahman and Islam, 2013).

In our study, the plant extracts had different synergistic ability to inhibit the growth of microorganism depending on the method of extraction. Plants

Table 3 : Antibiogram for Gram negative bacteria.

Antibiotic	*E.coli*		*K. pneumonia*		*Proteus* sp		*Shigella* sp		*N.gonorrhea*	
	Inhibition zone (mm)	Antimicrobial susceptibility	Inhibition zone (mm)	Antimicrobial susceptibility	Inhibition zone (mm)	Antimicrobial susceptibility	Inhibition zone (mm)	Antimicrobial susceptibility	Inhibition zone (mm)	Antimicrobial susceptibility
Penicillin	0	R	0	R	0	R	0	R	0	R
Ampicillin	0	R	0	R	0	R	0	R	0	R
Oxacillin	0	R	0	R	0	R	0	R	0	R
Azithromycin	0	R	0	R	0	R	0	R	0	R
Meropenem	23	S	0	R	18	R	14	R	15	R
Tobramycin	0	R	0	R	0	R	0	R	0	R
Gentamicin	0	R	0	R	0	R	0	R	0	R
Tetracyclin	0	R	0	R	0	R	0	R	0	R
Ciprofloxacin	25	I	0	R	0	R	10	R	13	R
Levofloxacin	0	R	0	R	0	R	0	R	0	R
Chloramphenicol	18	S	18	S	20	S	20	S	20	S
Clarithromycin	0	R	0	R	0	R	0	R	0	R

antimicrobials have been found to be synergistic enhancers in that though they may not have any antimicrobial properties alone, but when they are taken concurrently with standard drugs they enhance the effect of that drug (Chanda and Rakholiya, 2011). The synergistic effects resulting from the combination of antibiotics with plant extracts were documented in the literatures (Aburjai *et al*, 2001). Drug synergism between known antibiotics and bioactive plant extracts is a novel concept and could be beneficial (synergistic or additive interaction) or deleterious (antagonistic or toxic outcome) (Adwan and Mhanna, 2008).

CONCLUSION

1. It could be finally concluded that the multi-drug resistant Gram positive and gram negative bacteria were sensitive to most of the tested plant extracts due to their strong antimicrobial activities.
2. Suggesting that very small amount of the Plant extracts of *Anastatica hierochuntica* have great potential as antimicrobial compounds against pathogenic bacteria. Thus, they can be used in the treatment of endometritis caused by resistant microbes to antibiogram agent.
3. The synergism effect from the association of chloramphenicol antibiotic with plant extracts against resistant bacteria leads to new choices for the treatment of infectious diseases. This effect enables the use of the respective antibiotic when it is no longer effective by itself during therapeutic treatment.
4. Methanolic plant extracts were showed antimicrobial and synergistic activity with antibiotics better than aquatic extracts.

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