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The Antibacterial Effect of Momordica Charantia (Karela) Plant Extracts and Their Combination with Antibiotic Drugs

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Abstract- The aim of the study was to assess the antibacterial effect of Momordica charantia plant extracts and of extracts in combination with antibiotic drugs against Escherichia coli and Staphylococcus aureus. The aqueous and ethanolic extracts of Momordica charantia leaves were prepared using water reflux and soxhalation respectively. The antibacterial activities of extracts were evaluated using the well diffusion method and the inhibitory zones were recorded in millimeters. The effect of combination of Momordica charantia plant extracts and antibiotics drugs was assessed using well diffusion method.The results of this study showed that ethanolic extract of Momordica charantia exhibited better antibacterial activity against E. coli and. S. aureus than the aqueous extract. Also synergistic antibacterial effect was observed by both aqueous and ethanolic extracts of Momordica charantia with antibiotic drugs cefotaxime and ceftriaxone. However ethanolic extract in combination of both the anitiobiotic drugs showed better antibacterial results than that with aqueous extracts . Thereby, our results indicate the possibility of using aqueous and ethanolic extracts of Momordica charantia in the treatment of bacterial infections. Also these extracts showed promising results to be used in combination with synthetic antibiotics to give synergistic antibacterial effect. However there is need for clinical studies to determine the real effectiveness and potential toxic effects in vivo. These results revealed the importance of plant extracts when associated with antibiotic drugs in control of bacteria.

I. INTRODUCTION

The development of bacterial resistance to presently available antibiotics has necessitated the need to search for new antibacterial agents. Different antibiotics exercise their inhibitory activity on different pathogenic organisms (Chanda and Rakholiya, 2011). Multiple drug resistance in human pathogenic microorganisms has been developed due to indiscriminate use of commercial antibacterial drugs commonly used in the treatment of infectious diseases. The development of antibiotic resistance is multifactorial, including the specific nature of the relationship of bacteria to antibiotics, the usage of antibacterial agent, host characteristics and environmental factors. This situation has forced scientists to search for new antibacterial substances from various sources as novel antibacterial chemotherapeutic agents, but the cost production of synthetic drugs is high and they produce adverse effects compared to plant derived drugs (Abiramasundari et al., 2011). These antibacterial substances are of natural origin, and it is thought that their influences on the environment are few and can be used as biological control agents. However, some medicinal herbs for some reasons have not found wider application and sometimes are referred as ‘forgotten plants’.Taking into account the increasing demand for natural ingredients that might be used as food additives, components of functional foods, preventing plant diseases and nutraceuticals as well as for other applications. It is reasonable to revise the ‘forgotten plants’ by assessing their applicability and benefits using modern scientific methods (Abdel Rahmanet al., 2011). Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Gislene et al., 2000). Gram positive bacteria such as Staphylococcus aureus is mainly responsible for postoperative wound infections, toxic shock syndrome, endocarditis, osteomyelitis and food poisoning (Benayache et al., 2001). Gram negative bacterium such as Escherichia coli is present in human intestine and causes lower urinary tract infection, coleocystis or septicaemia (Benhassaini et al., 2003; Benjilali et al.,1986).

Antibacterial resistance is the ability of bacteria to resist the effect of medication previously used to treat them. This resistance come through genetic mutation or several other factors. Some plants exhibit significant potency against human bacterial pathogens. However, at present, plant extracts are rarely used as antibacterials or as a systemic antibiotics and this may be due to their low level of activity, especially against gram-negative bacteria (Adwan and Mhanna, 2008).

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II. MATERIALS AND METHODS

A. Materials Bacteria

Strains of Staphylococcus aureus, and Escherichia coli were obtained from microbiology department of Smt. Kishoritai Bhoyar College of pharmacy Kamptee.

a). Culture Media and Chemicals

Nutrient agar media were obtained from Himedia laboratories Pvt. Ltd. Mumbai.

b). Antibiotics

Ceftriaxone and cefotaxim were obtained from Abbott and Alkem pharmaceuticals.

III. METHODOLOGY

A. Preparation of Plant Extract

a). Aqueous Extract of Momordica Charantia Plant Leaves.

For aqueous extraction, 20 g of air-dried powder was added to150 ml of distilled water and boiled on low flame of burner for 2 hours. The slurry was then filtered through layers of muslin cloth and centrifuged at 200 r.p.m. for 10 min. The supernatant was collected. This procedure was repeated twice .The supernatants were kept standing and collected after 2 hours ,combined together and concentrated to make the final volume one-fourth of the original volume (Parekh, and Chanda, 2006).

b). Ethanolic Extract Of Momordica Charantia Plant Leaves

The method of Jameela *et al*. (2011) was used to obtain ethanolic extracts in which 20 gram powdered leaves were extracted separately with 150 ml of 80 % ethanol as a solvent for 8 hours, using soxhlet equipment. Then the extract was filtered and allowed to evaporate in oven (45 ºC). The dried extract was dissolved in Dimethyl sulfoxide (DMSO) and stored in refrigerator for further use.

c). Preparation of Momordica Charantia Plant Extracts Standard Concentrations

One g of each aqueous extract and alcohol pre-prepared extract (each separately) was taken and the aqueous extract was dissolved in 5 ml sterile water, while alcoholic extracts were dissolved in 5 ml of Di Methyl Sulphoxide (DMSO). Thus 200 mg / ml of stock was obtained as a standard concentration of aqueous and alcoholic extracts.

d). Evaluation of Antibacterial Activity of Cefotaxime And Ceftriaxone

According to Obeidat *et al*. An inoculum suspension was swabbed uniformly to solidified nutrient agar for bacteria, and the inoculum was allowed to dry for 5 min. Holes were made in the seeded agar. Aliquot of 10µl of each antibiotic i.e. cefotaxime (20 mg/ml) and ceftriaxone (40 mg/ml) was added into separate well on the seeded medium and allowed to stand on the bench for 1 h for proper diffusion and thereafter incubated at 37oC for 24 h. The resulting inhibition zones were measured in millimeters (mm).

e). Evaluation of Antibacterial Activity of Extracts of Momordica Charantia Leaves

The methodology for evaluation of antibacterial activity of extracts of momordica charantia leaves were same as the evaluation of antibacterial activity of cefotaxim and ceftriaxone with the concentration of 200 mg/ml of extracts.

f). Evaluation of Antibacterial Activity of Extracts of Momordica Charantia

leaves in combination with the antibiotics, Cefotaxime And Ceftriaxone Each bacterium (*Escherichia coli and S aureus*) was inoculated on the surface of nutrient agar plates. The antibiotics (Cefotaxime And Ceftriaxone) ,10 µl each was added in to separate wells ,Subsequently 10µl of plant extracts (Aqueous and Ethanolic) were added in to wells (at a concentration of 200mg/ml), to check the antibacterial effect of the plant extracts in combination with antibiotics. The plates were incubated at 37º C for 24 h. The diameters of clearing zones were measured.

IV. RESULTS

A. Evaluation of Antibacterial Activity of Cefotaxim and Ceftriaxone

The results of antibacterial activity by well diffusion method showed that the ceftriaxone showed the greater antibacterial activity as compared to cefotaxim against *Staphylococcus aureus* as well as cefotaxim exhibited greater antibacterial activity compared to ceftriaxone against *Escherichia coli.* As showed in table no.1 and fig. 1 and fig 2.

Microorganism / Antibiotics	*Staphylococcus aureus*	*Escherichia coli*
	Inhibition zone (mm)	Inhibition zone (mm)
Cefotaxim(c_1)	27.25	31.25
Ceftriaxone(c_2)	30	28
Control	……	……

Table 1. Evaluation of Antibacterial Activity of Antibiotics Cefotaxim and Ceftriaxone Against.

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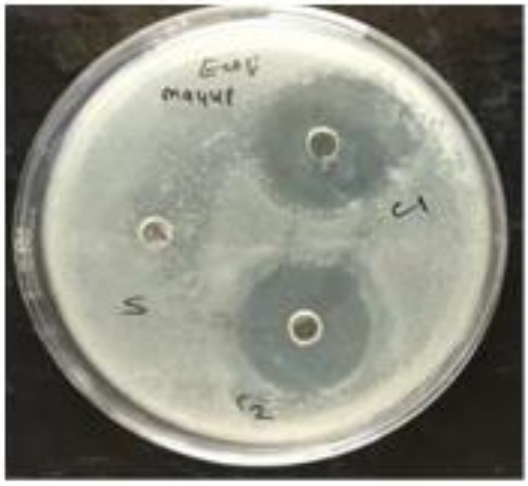

Fig. 1 Inhibition Zones (Mm) of Cefotaxim and Ceftriaxone Against *E. Coli.*

Fig.2 Inhibition Zones (Mm) of Cefotaxim and Ceftriaxone Against *S. Aureus.*

B. Evaluation of Momordica Charantia Plant Leaves Extracts Antibacterial Activity.

The result in table 2 and fig.3 and fig.4 shows the antibacterial activity of momordica charantia plant leaves extracts by well diffusion method. Ethanolic extract showed the greater antibacterial effect against *S. aureus* as well as against *E. coli* with a zone of inhibition of 12.5 mm and 15.2 mm respectively.

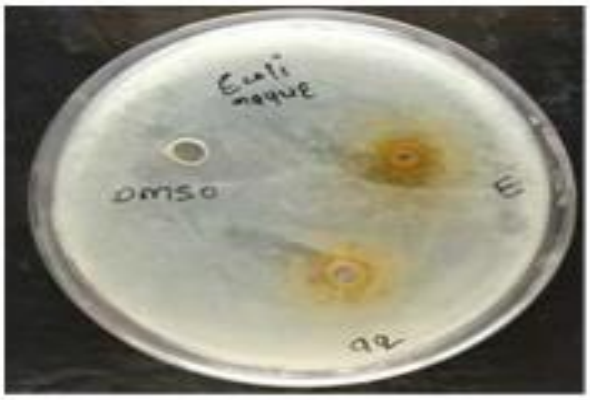

Fig.3 The Effect of *Momordica Charantia* Aqueous and Ethanolic Extract Against *E. Coli* .

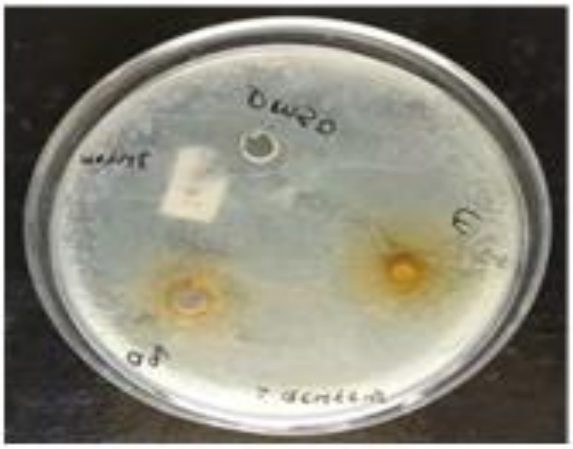

Fig.4 The Effect *of Momordica Charantia* Aqueous and Ethanolic Extract Against *S.Aureus*..

Microorganism / Extract	*Staphylococcus Aureus*	*Escherichia coli*
	Inhibition zone (mm)	Inhibition zone (mm)
Aqueous(aq)	11	12.75
Ethanol(E)	12.5	15.2
Control	……	……

Table 2. Antibacterial Activity of Momordica charantia Plant leaves extracts on *E. coli* and *S. aureus* by well diffusion method.

C. Evaluation of Antibacterial Activity of Aqueous Extracts With Antibiotics

As shown in table 3 and the following fig 5,6,7 and 8 Cefotaxime when used in combination with aqueous extracts of the leaves of *Momordica charantia* showed better antmicrobial effect (inhibitory zone 32.5 mm) . However the zone of inhibition(26.5 mm) obtained by using ceftriaxone with aqueous extract of the leaves of momordica charantia was smaller than ceftriaxone alone (28 mm) against *E. coli* but against *S. aureus* the zone of inhibition of both the drugs Cefotaxime and ceftriaxone when used in comination with aqueous extract of the leaves of *Momordica charantia* were greater measuring 36.5mm and 32.5 mm. than cefotaxime and ceftriaxone alone respectively.

D. Evaluation of Antibacterial Activity of Ethanolic Extract With Antibiotics

As shows in table 3 the following fig 5,6,7 and 8 cefotaxime when used in combination with ethanolic extracts of the leaves of *Momordica charantia* showed better antibacterial effect than ceftrtriaxone against *S. aureus* as well as against *E. coli* and the results suggested that cefotaxime and ceftriaxone exhibited antibacterial activity in combination with ethanolic extract of the leaves of *Momordica charantia* than alone.

Microorganism / Extract with antibiotic	*Staphylococcus aureus*	*Escherichia coli*
	Inhibition zone (mm)	Inhibition zone (mm)
Cefotaxime alone	27.25	31.25
Aqueous with cefotaxim(C_1aq)	36.5	35.3
Ceftriaxone alone	*30*	*28*
Aqueous with Ceftriaxone(C_{2aq})	32.5	26.5
Cefotaxime alone	27.25	31.25
Ehanolic with cefotaxim(C_1E)	36.5	33.25
Ceftriaxone alone	30	28
Ehanolic with Ceftriaxone(C_2E)	33	32.5
control	……..	……

Table 3-Antibacterial Effect of Combination of Antibiotics With Aqueous And Ethanolic Plant Leaves Extract of *Momordica Charantia* Against *E. Coli* And *S. Aureus.*

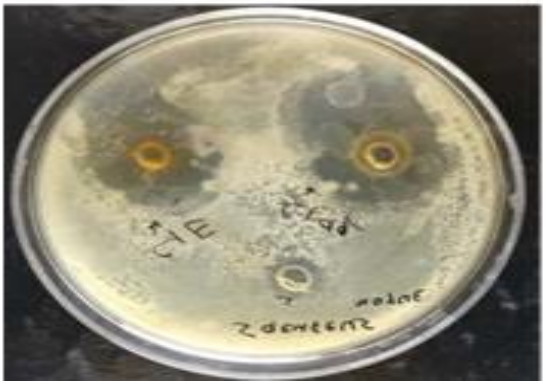

Fig. 5 Evaluation of Antibacterial Activity of Aqueous And Ethanolic Extracts of Momordica Charantia in Combination With Cefotaxim Against *S. Aureus.*

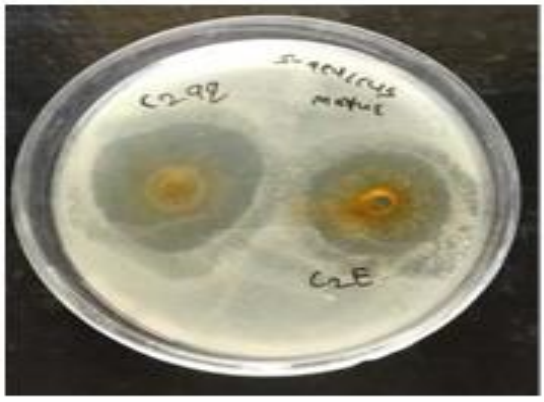

Fig. 6 Evaluation of Antibacterial Activity of Aqueous and Ethanolic Extracts of Momordica Charantia in Combination With Ceftriaxone Against *S. Aureus.*

Fig.7 Evaluation of Antibacterial *Activity.*of aqueous and ethanolic extracts of *Momordica charantia* in combination with cefotaxim against *E.coli*

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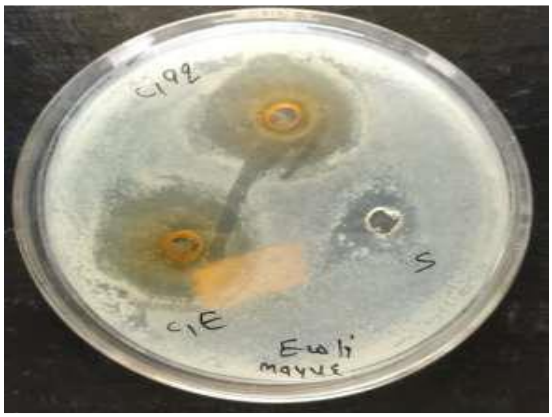

Fig.8 Evaluation of Antibacterial Activity of Aqueous and Ethanolic Extracts of Momordica Charantia in Combination With Ceftriaxone Against E.Coli.

V. CONCLUSION

On the basis of the antibacterial assay of this study *E. coli* was found to be the more susceptible to the employed Momordica charantia plant leaves extracts than *S.aureus.* Also the combination of Momordica charantia plant leaves extracts and antibiotics cefotaxime and cefotaxime exhibited the better synergistic effect with ethanolic extract against *E. coli* and *S. aureus* . The aqueous extract of Momordica charantia plant leaves did not exhibited any synergistic effect against *E. coli* with ceftriaxone. In addition this study shows that the cefotaxime shows the better synergistic effect with Momordica charantia plant leaves extracts than the ceftriaxone

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