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IN VITRO EVALUATION OF ANTIMICROBIAL ACTIVITY OF ROOTS EXTRACTS OF *Capparis spinosa* L. AND IMPORTANT MEDICINAL PLANT

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ABSTRACT

Present work was aimed to study the antimicrobial potential of water, methanol, ethanol and ethyl acetate extracts of roots of an important medicinal plant *Capparis spinosa* against *Escherichia coli*, using disc diffusion method. It was noted that extracts taken from dried and powdered roots of *Capparis spinosa* in four different solvents revealed antimicrobial activities at all the six different concentrations. Maximum inhibitory zone 19.72 mm was obtained at 6.0 mg/ml concentration of the extract taken in Ethyl acetate, followed by 18.54 mm at the concentration 4.0 mg/ml. At the similar concentrations, extracts taken in Ethyl alcohol had 18.36 mm and 17.36 mm inhibitory zones respectively. This was followed by the extracts taken in Methyl alcohol which was 16.56 mm and 15.20 mm respectively. Here lowest inhibitory zones were observed at all the six concentrations, when the extract was taken in distilled water. Therefore, in the solvent Ethyl acetate was superior followed by ethyl alcohol, methyl alcohol. Similarly, 6.0 mg/ml concentration of all the extracts gave better response than the rest of the concentrations used here. So the root extract of *Capparis spinosa* acts as natural antimicrobial agent. Therefore, they may be utilized in drug for the treatment of disease caused by the above test organism.

KEYWORDS: Antimicrobial Activity, *Capparis spinosa*, Solvent, Inhibitory Zone, Disc Diffusion Method, Bacterial Disease, Natural Antimicrobial Agent

Antibiotics are the most important classes in therapeutic agents and have given an enormous impact on both of life expectancy and improve quality of life. After discovery of penicillin, several other antibiotics have been isolated from different microorganisms and being used as a therapeutic agent. However, due to irregular consumption of antibiotics, there are emergences of antibiotic resistant microorganism towards synthetic antimicrobial agents. We are now getting multi drug resistant bacterial strains.

Therefore, it became essential to research on an alternative mode of treatment or to find an alternative antimicrobial agent to control the multi drug resistant microbes.

Plant products play an important role in the health care systems of more than 50% of the population, residing all over the world. Secondary metabolites, which plants employ to defend themselves against bacteria, fungi and other pathogens, can be used almost the same way in medicine to treat different infections. Plants usually produce secondary metabolites in complex mixture, whose composition show substantial differences between developmental stages and organs. Most plants accumulate phenolic compounds (Flavonoids and tannins), that are regularly accompanied by terpinoids (Monoterpene, Sesquiterpenes, triterpene or Saponins). Much research has been done in crude extracts, fractions, essential oils and also isolated compounds to search antimicrobial compounds. Many papers have been published on the topic such as antimicrobial activity of different extracts taken from different parts of medicinal plants.

Traditional approaches to find new antimicrobial drugs are not sufficiently successful any more due to the rapid resistance development against them. (Abreu *et al*; 2012). Plant materials are demonstrated to be one of the most promising sources (Abreu *et al*; 2012).

In the present study, *Capparis spinosa* L. was selected for the antimicrobial activity. *Capparis spinosa* L. also known as the caper bush, belongs to family *Capparaceae*, is a perennial winter deciduous species. Flower is large white to pinkish in colour. It can grow in different kinds of habitat. Plant derived antimicrobials are also considered to be safer compared with synthetic compounds because of their natural origin (Rajesh *et al*; 2010; Upadhyaya *et al*; 2014). Rates (2001) reported that quarter part of current medications is derived from compounds of plant origin. Ahmad and Beg (2001); Petrosyan *et al*; (2015) reported that plant derived compounds could have other target sites than traditional antimicrobials and subsequently having different mechanism of action against microbes. Aneja *et al*; (2010); Rajesh *et al*; (2010); Cowan (2012); Upadhyaya *et al*; (2014) reported that plant secondary metabolites

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have different groups such as Phenolics, and poly phenols (flavonoids, quinines, tannins, coumarins,), terpenoids, alkaloids, lectins and polypeptides. All these compounds have antimicrobial properties, due to this phytoextracts acts against the microbes. There are different modes of actions of these chemicals as has been described by Gonovyan *et al*; (2017).

We have a long list of medicinal plants but only few have been evaluated *in vitro* for their antimicrobial activities *in vitro* (Borris 1996; Petrosyan *et al*; 2015). From the ancient times, people started to use plant materials to treat infectious disease, to heal the cuts and wound without knowing about the causative agents present among the plants. (Rios and Recio; 2005).

There are several research papers, where antimicrobial activities of phytoextracts have been reported. Some of them may be mentioned here, such as Ali *et al*; (1998); Sokmen *et al*; (1999); Kalia *et al*; (1999); Thongson *et al* ;(2004); Elizabeth (2005); Chaudhary and Tariq (2006); Khokra *et al*; (2008); Rajshekaran *et al*; (2008); Rupa shree *et al*; (2008); Satish *et al*; (2008); Aneja & Joshi (2009); Kumar *et al*; (2009); Pal & Chattopadhyaya (2009); Darwish and Aburajai (2010); Das *et al*; (2010); Karsha & Lakshami (2010); Lalitha *et al*; (2010); Pratima and Sunder (2010); Ababutain (2011); Alagesa (2011); Vijaya lakshami *et al*; (2011); Brindha *et al*; (2012); Selvamohan *et al*; (2012); Akrayi and Zirak *et al*; (2013); Khan *et al*; (2013); Shanta and Maliki (2013); Kumar *et al*; (2014); Sadia *et al*; (2014); Majeed and Ghizawi (2016); Ramin and Razavi (2016); Neha *et al*; (2017); Mandhar *et al*; (2019). In the present work *Capparis spinosa* L. was selected based on its applications by locals in general and the rural people in particular. Here crude extracts in four different solvents were tested for its antimicrobial activity *in vitro.*

MATERIALS AND METHODS

Pure culture of *Escherichia coli* was made available from the microbial biotechnology laboratory, University Department of Botany, B.R.A., Bihar University, Muzaffarpur, Bihar. The culture was maintained on LB medium.

Composition of L.B. Medium:

5.0 g Tryptone,

2.5 g Yeast extract

5.0 g NaCl

500 ml distilled water

In a 2000 ml Erlenmeyer flask, 1000 ml distilled water was taken. Now 25 g of L.B. medium powder was weighed and taken in the above flask. Now 1.5% (15g) of bacteriological agar powder was added in the flask. The mouth was covered with aluminum foil and sealed with autoclavable tape. The medium was autoclaved at 15 lb pressure for 20 min. the pressure was released and the flask was taken out carefully. Now pre-sterilized Petri plates were placed on the working table. The covers of the plates were removed. Semi gelled medium was poured in the Petri plates and the covers were replaced.

These plates were allowed to cool and 10 plates were stacked together with the help of rubber bands. Plates were stored in freeze and they were placed upside down. These cultures plates were used for the maintenance of *E. coli* in the laboratory.

Preparation of Muller Hilton Medium

Ingredients

Beef infusion form: 300 g/l

Casein hydrolysate: 17.5 g/l

Starch 1.50g/l

Distilled water:- 1000 ml

pH was adjusted to 7.3 by adding 1 N KOH drops.

Above medium was Muller Hilton broth.

When semisolid medium was used then 17 g bacteriological agar powder was added 1 liter, medium was dispensed into 250 cc, culture flaks at the rate of 40-50 ml per flask. After proper plugging, with the cotton plug, covered with muslin cloth, the plugs were wrapped with Aluminum foil to avoid wetting during autoclaving. Autoclaving was done at 15 lb pressure for 20 minutes. Above culture flaks were allowed to cool at room temperature. Inoculation was done in the aseptic conditions of laminar air flow chamber. During inoculation, precautions were taken to prevent cross contamination.

Extraction of Phytochemicals

Capparis spinosa was located in the University campus. With the help of knife, roots were excised from the plant after digging the soil. These roots were brought in the laboratory and washed properly to remove the adhering soil particles. Now they were cut into small pieces and placed in a well cleaned plastic bag. These roots were dried under shade in the laboratory. Well dried

pieces of roots were ground to powder with the help of iron mortar and pestle. Sometimes grinder was also used to obtain fine powder. Above powder was passed through muslin cloth to remove some large pieces of the roots. Now the poser was stored in a well cleaned glass bottles. The bottle was made air tight with crew cork. It was labeled.

Preparation of Extract

30 g of powder of roots of *Capparis spinosa* was weighed. Above powder was soaked separately in four different solvents such as:

i. Distilled water
ii. Methanol 70%
iii. Ethanol 70%
iv. Ethyl acetate 70%

200 ml of each solvent was taken in four different 500 ml flask. In the above solvent, 30 g powder was soaked separately at 10:1 solvent solute ration v/w and mixed properly. Flasks were stored for 48 hours at room temperature. Above flasks were stirred at regular intervals with pre-sterilized glass rod. Above powder soaked in four different solvents were filtered separately through double layered of muslin cloth.

The filtrates were centrifuged at 4000 rpm for 15 minutes and finally filtered through Whatman filter paper no.-42. All the four filtrates were taken separately in four conical flasks. The residue were further soaked in fresh respective solvents in the ratio as mentioned above and left for 48 hours. This step was repeated three times in order to gain maximum extraction of active compounds. All the extracts of each solvent were pooled together. Aqueous extract was evaporated by heating while the methanolic, ethanolic and extract in ethyl acetate were evaporated in water both at 40^0C. The residues so obtained were scratched carefully and preserved in the air tight bottles labeled separately.

Each scratched residue was dissolved in the same solvent in which they were extracted. From the stock solution the concentrations were adjusted as 0.5 mg, 1.5 mg, 2.0 mg, 4.0 mg and 6.0mg /ml.

Antibacterial Activity of the Extracts

Bacterium *E. coli* was maintained in the laboratory. Form the above culture inoculums was taken and plated on Mueller-Hinton agar medium. This was cultured at 37^0C over night. From the above culture plates bacteria were harvested using 5 ml of saline water. The plate was rubbed with brush after adding 5 ml saline water. The turbidity was measured at 600 nm. The bacterial suspension was diluted in such a way that absorption came to 0.5. This is called Mc Farland standard which equals to $1\text{-}2X10^8$ CFU/ml (Colony forming unit). Above inoculums then was diluted in 1: 100 ratio, so that $1X10^7$ CFU/ml concentration was obtained. This was stored at 4^0C until use. The diluted inoculums were cultured on solid medium to verify the absence of contamination and dilutions made above.

60 ml of partially solidified Mueller-Hinton agar medium was poured in 150 mm Petri dish. This was covered with lid in such a way that within 5-6 minutes the surface became dry. These plates were stored at 4^0C before use. A sterile Swab was dipped into the inoculums containing tube. Then swab was rotated against the side of the tube to remove extra fluid. The swab streaked on the culture plate having Mueller-Hinton agar medium. It was done thrice.

The plate was rotated at 60^0C degrees each time to ensure an even distribution of the inoculums. Then the swab was discarded. 6 mm paper disc was properly impregnated with the phytoextracts taken in different solvents separately. In one plate all the discs were containing one concentration only. The discs were placed at distance and pressed with the tip of a sterilized forceps, to ensure complete contact with the agar surface. The plate was placed for 1 hour at low temperature and then incubated in culture room at 37 ± 1^0C. The zone of inhibition was measured, observing it form the back side of the plate with the help of a scale. The length of paper disc was deducted from the total length to determine the inhibition zone. In this way experiments were done for all the concentrations of afore mentioned four solvents. All experiments were repeated thrice and mean of the data was tabulated in Table-1.

Three antibiotics Ampicillin, Chloroamphenicol and Ciprofloxin in 5, 25, 50, 100 and 200µg/disc were tested for antibacterial activity. The data have been presented in Table-2.

RESULTS

Antibacterial activity extracts of roots of *Capparis spinosa* extracted in four different solvents at different concentrations was studied on *E. coli* through disc diffusion technique. The concentration of crude extracts was 0.5-6.0 mg/disc. Mean of the data was represented by the Table-1. From the table it may be noted that crude aqueous extract of roots had maximum inhibitory zone 14.76 mm at 6 mg/ml per disc and

minimum 6.50 mm at 0.5 mg/ml per disc. Here the next highest inhibitory zone 13.42 was obtained at 4.0 mg/ml/disc. Crude extracts in 70% Methyl alcohol revealed highest inhibitory zone 16.56 mm at 6.0 mg/ml/disc followed by 15.2 mm at 4.0 mg/l/disc. Here minimum inhibitory zone 8.76 mm was obtained at 0.5 mg/ml/disc. Crude extracts in 70% Ethyl alcohol had maximum inhibitory zone 18.36 mm at 6.0 mg/l/disc followed by 17.36 mm at 4.0 mg/l/disc. Here minimum inhibitory zone 9.20 mm was noted at 0.5 mg/ml/disc. Crude extracts extracted in 70% Ethyl acetate had maximum inhibitory zone 19.72 mm at 6.0 mg/l/disc followed by 18.54 mm at 4.0 mg/ml/disc. The minimum inhibitory zone 9.86 mm was obtained at 0.5 mg/ml/disc here. It may be noted that crude extracts of roots of *Capparis spinosa* extracted in four different solved revealed antibacterial activities but there were variations in the zone of inhibition among the extracts.

Table 1: Showing antimicrobial activities of root extracts of *Capparis spinosa* L. taken in four different solvent at six different concentrations as indicted by inhibitory zone

Inhibition zone (in mm)	Solvent water	Methanol 70%	Ethanol 79%	Ethyl acetate 70%	Concentration
	6.50	8.76	9.20	9.86	0.5
	8.75	10.45	11.78	13.30	1.0
	10.34	12.18	13.85	14.88	1.5
	12.20	14.56	15.20	17.40	2.0
	13.42	15.20	17.36	18.54	4.0
	14.76	16.56	18.36	19.72	6.0

Table 2:

Inhibition	Ampicillin	Chloroamphoid	Ciprofloxin	Concentration (µg)
	10.46	10.55	16.36	5.0
	12.35	14.46	17.54	25.0
	14.28	17.38	22.65	50.0
	18.15	18.20	23.48	100.0
	19.28	22.15	24.81	200

DISCUSSION

Different workers have used crude extracts of different medicinal plants to study its antibacterial activity against different bacterial pathogens. Okeke *et al*; (2001) evaluated antibacterial activity of root extracts of Landolphia owerrrience against four different bacteria such as *Staphylococcus aureus, Pseudomonas aeruginesa, Escherichia coli* and *Salmonella typhi*. They also concluded that ethanolic extract had maximum inhibitory zone than that of the aqueous extracts. Darwish and Aburjai (2010) evaluated antibacterial activity of ethnomedicinal plants used in folklore medicine against *Escherichia coli*. They combined antibiotic and plant extract and reported that, even multi drug resistant *E. coli* was affected due to synergistic effects of these two. This strategy was named as "herbal shotgun" or "synergistic multitarget effects". Bhalodia and Shukla (2011) studied antibacterial and antifungal activities of leaf extracts of *Cassia fistula*, an ethnomedicinal plant. They concluded that extracts of *Cassia fistula* revealed antibacterial and antifungal activities. They concluded that hydroalcoholic extract of *Cassia fistula* were more effective than that of the standard drugs. They further reported that antibacterial activity of the extracts increased along with increasing concentrations of the extract. Findings of present work are therefore, in agreement with the above findings. Vijayalakshami *et al*; (2011) reported *in vitro* screening of *Catharanthus roseus* alkaloid for antibacterial activity. They reported that even at minimum concentration 10µg/ml exhibited inhibition bacteria in the culture up to 9 mm in diameter. Selvamohan *et al*; (2012) reported antibacterial activity of selected medicinal plants against some selected human pathogenic bacteria. They used seven different plant species and reported that alcoholic extracts of the plants had maximum antibacterial activity than the aqueous one. In this way the findings of the present work corroborate with the above findings as here also alcoholic extracts revealed better impact on bacterial growth than that of the aqueous one. Antibacterial activity of different plants extracts have also been reported by Kumar *et al*; (2014); Rajesh *et al*; (2014). All these workers reported that

antibacterial activity was directly related with the concentrations of the extracts and solvent used.

Al-Majeed and Al-Ghizawi (2016), isolated and identified alkaloids form the extracts of *Capparis spinosa* L. buds and studied its antibacterial activity. They concluded that the diameter of inhibitory zone was concentration dependent. Ginovyan *et al*; (2017) reported antimicrobial activity of some plant materials used in Armenian traditional medicine. They concluded that extracts taken from different parts of the medicinal plants possessed antimicrobial activity against both the Gram-positive and Gram-negative strains.

They also concluded that alcoholic extracts were superior to the aqueous extracts. Hina *et al* (2019); Mandhar *et al*; (2019); Rajesh *et al*; (2019), reported antibacterial activity of extracts taken from different medicinal plants. They also concluded that extracts of different plant species had different quantum of inhibition of bacterial growth even at the same concentrations. They also reported that alcoholic extracts had maximum inhibitory zone than that of the aqueous extracts. All these reports are in agreement with the findings of the present work.

CONCLUSION

Extracts taken from the roots of *Capparis spinosa* in four different solvents revealed antimicrobial activities at different concentrations. However, maximum inhibitory zone was found at all the concentration of the extracts when the solvent was Ethyl acetate. This was followed by Ethyl alcohol-Methyl alcohol. Here lowest inhibition zone was found in the aqueous extract.

This may be correlated with the facts that the different secondary metabolites might have dissolved in the alcoholic solvent in comparison to the aqueous one. Similarly, inhibitory action was concentration dependent. So herbal extracts may be used for the control of microbial infection as they are natural products and had no side effects.

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