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Exploration of Anti-microbial activity of copacīnī (*Smilax china*) root on various pathogens causing Urinary Tract Infection – an In vitro study

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Abstract –

Introduction

The present study was carried out with an objective to investigate the antimicrobial potentials of various extracts of copacīnī (*Smilax china*) fruits on common uropathogen strains.

Material and Methods

Aqueous, ethanol, chloroform, petroleum ether extracts of *Smilax china* L. were evaluated for potential antimicrobial activity against certain uropathogen strains. The antimicrobial activity was determined in the extracts using agar well diffusion method. The antibacterial activities of extracts (5%, 10% and 15% w/v) of *Smilax china* were tested against *Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumonia* and *Enterococcus faecalis.* Zone of inhibition of extracts were compared with that of standard drug Azithromycin 1 % w/v(Positive control) and DMSO(Negative control) for antibacterial activity.

Observations and results

As compared with standard drug, the results revealed that all extracts possesses anti bacterial activity but ethanol extract was found to have maximum activity against all pathogens taken in this study. Maximum antibacterial activity was reported against *Proteus mirabilism* by all extracts. The phytochemical analyses of the plants were also carried out which was found to be similar to standard values of API.

Conclusion

In vitro findings of this study confirmed urobactericidal activity of *Smilax china* on common uropathogens.

Keywords: *Smilax china*, in vitro antimicrobial activity, UTI, uropathogens, in vitro study

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Introduction –

Urinary tract infections (UTIs) are common, recurrent infections that can be mild to life-threatening. It is a condition in which any part of the urinary tract (urethra, bladder, ureter, and kidney) gets infected with bacteria or occasionally with fungus.[i] Both the sexes are prone to develop UTI with a female to male ratio of 2: 1 in patients older than 70 years as compared to a 50:1 ratio in younger population.[ii] UTIs can be classified as complicated or uncomplicated. Uncomplicated UTI is the most common type of infection and mainly occurs in the absence of functional or anatomical abnormalities within the urinary tract. The complicated one occurs in the presence of an abnormal urinary tract that increases susceptibility to infection. Among the uropathogen, *Escherichia coli* is the most common bacteria (75–90% of isolates) in both the community and hospital infections, whereas other pathogenic bacteria such as *Proteus mirabilis*, *Staphylococcus saprophyticus* (with particularly frequent isolation from younger female), *Enterococcus faecalis*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*.[iii]

As UTIs are caused mainly by bacteria, antibiotic therapy is an effective approach and reduces the duration of symptoms typically within 3 to 7 days. Development of UTI in hospitalized patients, especially those with an indwelling urinary catheter, extends the duration of patient stay and increases the complexity of patient treatment. In severe cases, urosepsis may occur which constitutes 25% of all adult sepsis cases, and is associated with an overall mortality of 20–40%.[iv] Thus, UTIs remains one of the important sources of significant morbidity and mortality which affect the quality of life of the affected patients. Beside this, another challenge is emergence of antibiotic-resistant bacterial pathogens which has resulted due to extensive use of antibiotics. The continued emergence of antibiotic resistance, together with our increasing understanding of the detrimental effects conferred by broad-spectrum antibiotic use on the health of the beneficial microbiota of the host, has underscored the weaknesses in our current treatment paradigm for UTIs. The recurrence of the urinary tract infections (UTI), following the antibiotic treatments suggests the pathogen's resistance to conventional antibiotics. This calls for the exploration of an alternative therapy.[v] Complementary and alternative medicine (CAM) has been recognized as an effective approach for the treatment of infection by antibiotic-resistant bacteria.[vi] The present study was carried out with an objective to investigate the antimicrobial potentials of various extracts of copacīnī (*Smilex china*) on common uropathogen strains.

Material and Methods –

Test Sample

Test sample copacīnī (*Smilex china*) was purchased from local market and the sample was identified and authenticated by CSIR- National Institute of Science Communication and Information Resources, Raw material Herbarium and Museum, New Delhi (vide reference number NISCAIR/RHMD/Consult/2020/3708-09-1 to 5 on date 18/12/2018).

Preliminary Phytochemical Screening

The extracts were subjected to preliminary phytochemical testing to detect for the presence of different chemical groups of compounds. Air-dried and powdered plant materials were screened for the presence of saponins, tannins, alkaloids, flavonoids, triterpenoids, steroids, glycosides, anthraquinones, coumarin, saponins, gum, mucilage, carbohydrates, reducing sugars, starch, protein, and amino acids, as described in literatures.

Preparation of different extracts

The extraction of the *Smilax china* was carried out by using known standard procedures. Aqueous, Ethanol, Chloroform, Petroleum ether extracts were prepared by soxhlet extraction method and removal of solvent was done in rotary evaporator. 5%, 10% and 15 % w/v solution were prepared using Dimethyl sulfoxide (DMSO).

Test Microorganisms and Growth Media

Escherichia coli (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus mirabilis* (ATCC 12453), *Klebsiella pneumoniae*. (MTCC 4030), *Enterococcus faecalis* (MTCC 439) were chosen based on their clinical importance in UTI. The bacterial cultures were incubated for 24 hours at 37°C on nutrient agar and were then stored at 4°C. The bacterial strains were grown in Mueller-Hinton agar (MHA) plates at 37°C. The stock cultures were maintained at 4°C.

Antimicrobial Activity

Agar well diffusion method was chosen for in vitro anti microbial study as it is precise and reliable method. Autoclaved agar media (20 ml) was poured into each petri plate, followed by the swabbing of bacterial colony from the inoculums of the test microorganisms on prepared media plates. With the use of a sterile stainless steel borer, wells (with a diameter of about 5mm) were drilled into the plates. Using sterile syringes, plant solvent extracts were injected into the designated wells. The plates were then kept in an incubator at 37°C for 24 hours. Subsequently dimethyl sulphoxide-DMSO 0.1%- (the solvent used to reconstitute the test sample) was also poured to assess its activity, if any (as Negative Control) and standard antibiotic disc- Azithromycin 1 % w/v (Positive control) was placed in the same plate. All the plates were incubated at 37 °C for 24 h.

Determination of zone of inhibition method

Each plate was inspected after incubation for anti bacterial activity. The diameter of the well as well as the diameter of the zones of absolute inhibition were measured and recorded to the nearest whole millimetre. The experiment was done in triplicate, average diameter of the zone of inhibition was measured in millimeters by the help of the scale and then mean was calculated. The activity index was calculated from the mean of the three measurements by using formula as follows

Determination of the activity index[vii]

The activity index of the test samples extract was calculated as

$$\text{Activity index (AI)} = \frac{\text{Zone of inhibition of the extract}}{\text{Zone of inhibition obtained for standard antibiotic drug}}$$

Observations and Results –

Preliminary phytochemical screening

All the observed values of phytochemical study were corresponding with the standard values of API. Therefore the drug samples of copacīnī (*Smilax china*) used in this study was of desired quality.

Anti Microbial Activity

For screening antibacterial activity using Agar well diffusion method against clinical isolates of five uropathogens.[viii] Aqueous, Ethanol, Chloroform, Petroleum ether extracts were compared with the Positive Control (Standard drug Azithromycin 1 % w/v) and Negative control i.e. DMSO [Figures 1 to 5]. The results are presented in Table No. 1. & Table no.2

Table No. 1: Mean of ZOI(in mm) of different extracts of copacīnī (*Smilax china*) against *Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae, Enterococcus faecalis* with negative and positive control

Pathogen	Concentration	Zone of Inhibition (mm)				
		E. Coli	*P. aeruginosa*	*P. mirabilis*	*K. pneumoniae*	*E. faecalis*
Aqueous Extract of copacīnī	5% w/v	11	10	12	14	15
	10% w/v	14	13	15	16	17
	15% w/v	18	16	18	17	18
Ethanol Extract of copacīnī	5% w/v	12	14	15	16	14
	10% w/v	15	16	16	16	16
	15% w/v	20	21	19	21	19
Chloroform Extract of copacīnī	5% w/v	11	10	10	11	9
	10% w/v	12	13	13	12	11
	15% w/v	14	15	17	15	13
Petroleum ether Extract of copacīnī	5% w/v	12	10	11	10	11
	10% w/v	14	15	13	12	14
	15% w/v	16	17	18	15	16
Neg. Control		00	00	00	00	00
Positive Control		31	28	25	27	29

Table No. 2 : Activity index of different extracts of copacīnī (*Smilax china*)against trial pathogens in comparison to positive control

Pathogen	Concentration	Activity Index				
		E. Coli	*P. aeruginosa*	*P. mirabilis*	*K. pneumoniae*	*E. faecalis*
Aqueous Extract of copacīnī	5% w/v	0.35	0.36	0.48	0.52	0.52
	10% w/v	0.45	0.46	0.60	0.59	0.59
	15% w/v	0.58	0.57	0.72	0.63	0.62
Ethanol Extract of copacīnī	5% w/v	0.39	0.50	0.60	0.59	0.48
	10% w/v	0.48	0.57	0.64	0.59	0.55
	15% w/v	0.65	0.75	0.76	0.78	0.66
Chloroform Extract of copacīnī	5% w/v	0.35	0.36	0.40	0.41	0.31
	10% w/v	0.39	0.46	0.52	0.44	0.38
	15% w/v	0.45	0.54	0.68	0.56	0.45
Petroleum ether Extract of copacīnī	5% w/v	0.39	0.36	0.44	0.37	0.38
	10% w/v	0.45	0.54	0.52	0.44	0.48
	15% w/v	0.52	0.61	0.72	0.56	0.55

Aqueous extract of *Smilax china* showed significant biological activity against *K. pneumonia, E. faecalis* at 5% conc., against *P. mirabilis, K. pneumonia, E. faecalis* at 10% conc. and against all pathogens at 15% concentration. Ethanol extract of *Smilax china* was found to be biologically active against *P. aeruginosa, P. mirabilis, K. pneumonia* at 5% conc., against *P. aeruginosa, P. mirabilis, K. pneumonia, E. faecalis* at 10% conc. and against all pathogens at 15% concentration.

At 10% conc., Chloroform extract of *Smilax china* was found to be biological active against *P. mirabilis* whereas at 15% conc., activity index was significantly good against *P. aeruginosa, P. mirabilis, K. pneumonia.*
With activity index of 0.54 and 0.52, petroleum ether extract of *Smilax china* was significantly active against *P. aeruginosa and P. mirabilis.* At 15% conc. petroleum extract of *Smilax china* showed activity index of more than 0.50 against all pathogens.

Discussion –

Smilax china L., is a traditional herbal medicine mentioned in Ayurvedic classics for the treatment of various ailments and its therapeutic potential has been confirmed in earlier studies. The anticancer potential of *Smilax china* (rhizome, leaf, bark) extracts has previously been reported against various tumor cell lines including A549,[ix] HepG2 and MDA-MB-231[x] and HeLa cells.[xi] Extensive studies investigating *Smilax china* L. revealed its immunosuppressive,[xii] hypoglycemic,[xiii] antioxidant[xiv], and anti-inflammatory[xv] activities. Park et al. (2014) concluded that S. china methanol extract (SCME) has active compounds which have anti-obesity activities.[xvi] Another study reported that the ethyl acetate fraction of S. china rhizome has antipsoriatic activity.[xvii] Hypoglycemic activity of *Smilax china* has also been established in animal model. [xviii] Smilax china possesses various clinical potentials and many of them have been experimentally proven but its activity on uropathogens has not been explored meticulously. In one study antimicrobial activity of methanolic extract of *Smilax china* root against pathogenic microorganisms *Escherichia coli, Streptococci pyogens* and *Proteus mirabilis* was reported. [xix]

In Ayurveda, *Smilax china* has been mentioned as a drug having Mutravishodhani effect i.e.drug which cleanses urinary tract from various toxins, microorganisms etc. To explore its activity on uropathogens this study was carried on common uropathogens in which significant antibacterial activity was reported by all four extracts. The antibacterial activities of the extracts increased linearly with increase in concentration of extracts. It was discovered that increasing the concentration of extracts (5% w/v, 10% w/v, and 15% w/v) results in increased activity against all of the pathogens chosen for the study as measured by zone of inhibition. It's likely that the extract's secondary metabolite or chemical ingredient content will increase when the concentration of the extract rises. As a result, the ability to limit bacterial activity rises.
Maximum activity was shown by ethanol extract followed by aqueous and petroleum ether extract. The different activity could be a result of the different solvents utilized during the extract methods. The chemical composition of the extract and the permeability of microorganisms' membranes to chemical and metabolic processes may be responsible for variations in the extract's efficiency against certain microorganisms. Maximum activity was shown against *Proteus mirabilis* by all extracts.

Conclusion –

In vitro findings of this study confirmed urobactericidal activity of *Smilax china* on common uropathogens. As compared with standard drug, the results revealed that all extracts possesses anti bacterial activity but ethanol extract was found to have maximum activity against all pathogens taken in this study. Also maximum antibacterial activity was reported against *Proteus mirabilism* by all extracts.

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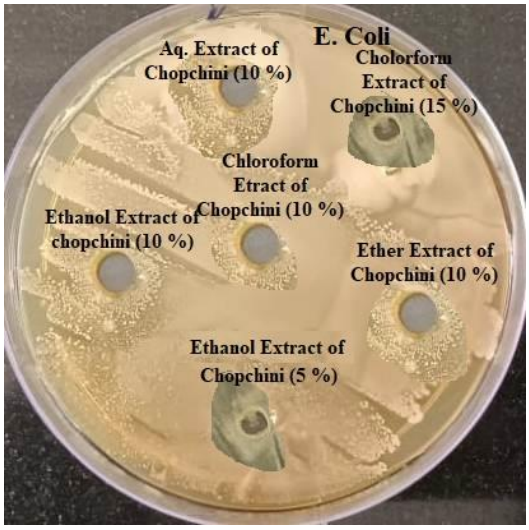

Fig.1

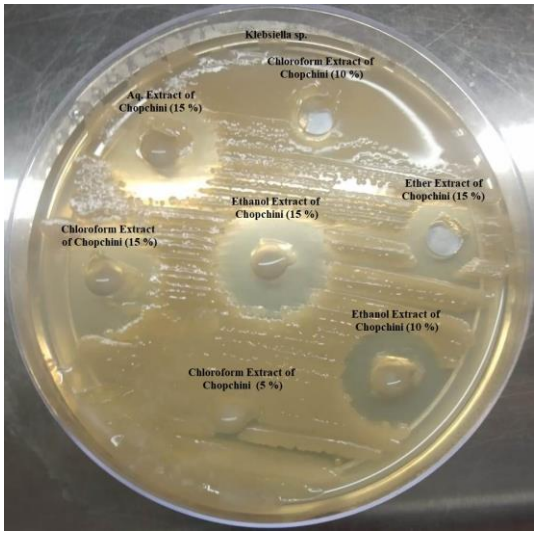

Fig.2

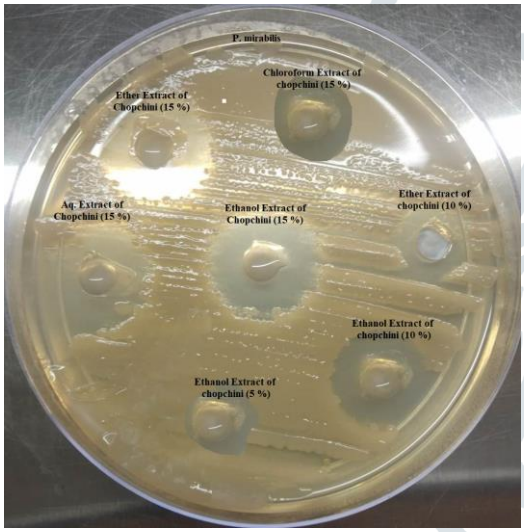

Fig.3

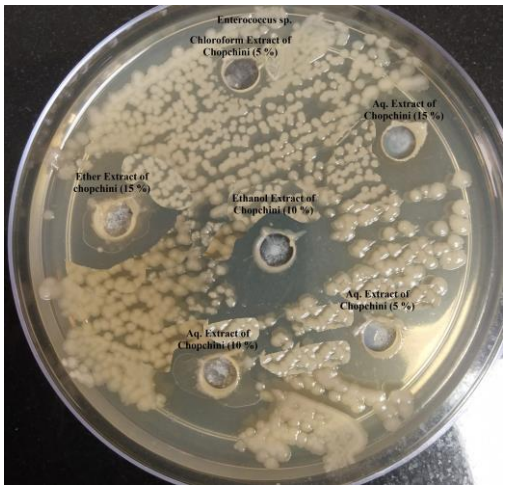

Fig.4

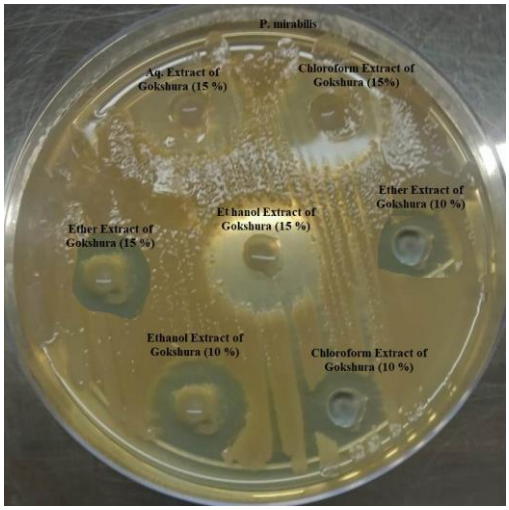
P. mirabilis
Aq. Extract of Gokshura (15 %)
Chloroform Extract of Gokshura (15%)
Ether Extract of Gokshura (10 %)
Ethanol Extract of Gokshura (15 %)
Ether Extract of Gokshura (15 %)
Ethanol Extract of Gokshura (10 %)
Chloroform Extract of Gokshura (10 %)

Fig. 5