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EXPERIMENTAL EVALUATION OF ANTIMICROBIAL ACTIVITY OF *KANTAKARI ARKA* (*Solanum xanthocarpum*)

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ABSTRACT

There are many antibacterial agents existing today but their improper usage leading to adverse effect or resistance to bacterial strains. It brings attention among the researchers towards Ayurvedic herbal drugs. In the present study, the Antibacterial activity of plant *Kantakari* (*Solanum Xanthocarpum*) was evaluated in vitro against selected human pathogenic microorganisms (*S. aureus, E.coli, S.pneumoniae, K. pneumoniae, M.pnumoniae, L.pneumoniae, P.aeruginosa and H.influenza* cultures) by measuring the zone of inhibition through well diffusion method. *Kantakari Arka* (distillation) and its Methanolic extraction was prepared by using *Panchangas* (*Mula, Twak, Patra, Pushpa & Phala*) of the plant *Kantakari.* The study reveals that, *Kantakari Arka* did not show antibacterial activity whereas Methanolic extraction of the same plant was effective in inhibiting the growth of *Staphylococcus aureus* and *E coli.*

Keywords: *Kantakari*, *Solanum Xanthocarpum*, *Panchangas*, Methanolic extraction.

INTRODUCTION

Kantakari (*Solanum xanthocarpum*) is one of the most potent drugs which is useful in different respiratory tract diseases. According to the classics, *Kantakari* is having *Tikta & Katu rasa, Dipana-pachana, Lagu-ruksha-ushna-sara guna,* indicated in *Kasa, Shwasa, Peenasa, Parshwa shoola, Khapa-vata hara, Krimighna and Hridroga hara*[1]. Currently *Kantakari* is used in much yoga like *Gritha, Avaleha, Kwatha, Choorna* etc. However, the palatability and its shelf life are the hindrance that arises in implementation of this formulation in prescriptions. There arises a need to overcome this drawback with modification of these dosage forms/ *Yogas* into a form which would render same results.

Arka Prakasha describes *Kalka*, *Churna*, *Rasa*, *Taila* and *Arka* as *Pancha prakara dravya Kalpana*. Among this *Arka* is said to be the most potent[2]. *Arka* is a liquid preparation obtained by distillation of certain liquids or of drugs soaked in water using the *Arka Yantra* or any convenient modern distillation apparatus[3]. This preparation was introduced to main stream pharmaceutical industry in the recent years. However, this preparation is to be taken with due regard because of its specificity in the preparation aspect with increased shelf life[4] and reduced dosage

in comparison with its counterparts. *Arka Kalpana* stability period is comparatively more than *Swarasa*, *Kalka*, *Kwatha*, *Hima*, *Phanta* and *Choorna*. *Arka Kaplana* is a unique dosage form with better palatability, high shelf life and maximum patient compliance.

Resistance of bacterial pathogens to existing antimicrobial agents possess problem to clinicians in the treatment of microbial infections. Search for newer and more effective compound is continuous process in drug discovery. In this direction plant derived antimicrobial compounds have attracted special attention among the researchers.

As *Arka* is most potent and suitable form for therapeutic administration, it is decided to prepare *Kantakari Arka*[5], analyse it chemically and finally screen its antimicrobial activity against eight common microbes which are responsible for the different systemic diseases viz, S.pneumoniae, K. pneumoniae, M.pnumoniae, L.pneumoniae, P.aeroginusa, S. aureus, E.coli and H.influenza.

Materials and Methods

Collection of Plant material

The drug *Kantakari* (*Solanum xanthocarpum*) required for the study was collected from village Kappalaguddi of Belgaum district, Karnataka, India in the prescribed month for collection of drug.

Processing of plant material

The collected *Panchangas* of *Kantakari* (*Solanum Xanthocarpum*) were cut into medium size pieces for quick drying. It was dried under shade for 18 days. The dried plant material was coarsely powdered in pulveriser having sieve with mesh size 9.

Preparation of *Arka*

Two samples of *Arka* were prepared according to *Ayurveda Sara Sangraha*[6] and *Arka* prakasha[7]. The *Kantakari churna* was soaked in sufficient quantity of water for overnight. Next day morning it was transferred to distillation apparatus and 8 parts of water (800ml) was added, a condenser was attached to it and closed properly. Appropriate temperature was given to distillation apparatus and 2/3rd (660ml) of the distillate was collected. According to *Arka prakasha*, 1 part drug was added with two parts of water. The method of preparation remains same as described above.

Preparation of Methanolic extract

Weighed quantity of *Kantakari* coarse powder was taken in 500 ml beaker and 50ml of methanol was added, stirred well and covered with aluminium foil. Then the beaker was kept on hot water bath at 50º C for 4 hours. After incubation period the extract was filtered with Whatman filter paper and the filtrate was collected in 50 ml beaker. Residue present over the filter paper was discarded and filtrate was taken for further use. Then the filtrate was kept at 80 ºC for few hours until the extract gets completely dried and turn into semisolid form. 100 mg of semi-solid crude extract was taken from the total yield and dissolved in 1 ml of methanol in an Eppendorf tube to make it 10mg/ml stock solution.

Procurement of bacterial strains

Bacterial strains used for evaluation of antimicrobial activity were procured from microbiology department, Skanda Life Sciences Pvt. Ltd, DSIR recognized R & D center, Bangalore, Karnataka, India.

Experimental study

A. Preparation of Nutrient agar-

5 gms of tryptic soy broth powder was dissolved in 125ml of purified water. Powder was completely dissolved by heating in water bath. Sterilized by autoclaving for 15 min at 121º c.

B. Preparation of inoculum:

Initially loop must be heated in spirit lamp up to red hot and was allowed to cool. With help of pipette 5ml of broth was transferred into the test tube. One colony of well isolated cultured study microorganisms which were in log phase was touched with the sterile loop and the growth was transferred into the test tube containing 5ml of nutrient broth. Then it was placed inside the incubator for 24hrs.

Cell suspension prepared on Tryptic soy broth for *S. aureus, E.coli, S.pneumoniae, K. pneumoniae, M.pnumoniae, L.pneumoniae,*

P.aeruginosa and H.influenza cultures.
The cell suspensions of all the cultures were adjusted to 1-2x 10^5cells/ml.

C. Grouping

1. Sample: Kantakari Arka
2. Standard: Ciprofloxacin
3. Control: Water

D. Determination of Antimicrobial of activity of Kantakari Arka by Agar well method[8]: Agar wells of 5mm diameter were prepared with the help of sterilized steel cork borer in each Petri dish. *Staphylococcus aureus, Escherichia coli, Streptococcus pneumonia, Klebsiella pneumonia, Mycoplasma pneumonia, Legionella pneumonia, Pseudomonas aeruginosa and Haemophilus influenza* (100 µl) was inoculated on Soybean casein digest agar plates (90 mm). Test compound *Kantakari Arka* (20µl) and ciprofloxacin (10 µl, 0.1 mg/ml) and water (20ml) were impregnated on 5mm well on agar plates. The plates were incubated at 37^0c for 24hrs in the incubation chamber. The zone of growth inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter.
Kantakari Arka did not show expected antibacterial activity against all the 8 bacterial strains. Hence, to know whether the chosen sample of *Kantakari* is having antimicrobial activity, its methanolic extraction was taken and screened for antibacterial activity against two organisms namely; Staphylococcus aureus & E.coli.

Determination of antibacterial activity of Methanolic extract of *Kantakari*

Staphylococcus aureus and *Escherichia coli, (*100µl) was inoculated on Soybean casein digest agar plates (90 mm). Test compound Methanolic extract (20µl), and ciprofloxacin (10 µl, 0.1 mg/mL) water 20(control group) were impregnated on 5mm well on agar plates. Treated *S. aureus* and *E. coli* plates were Incubated @ 37^0 C for 24 hrs. The plates were observed for zone of inhibition around the well.

Determination of Minimum Inhibitory concentration[9]

Mix the 90µl Methanolic extract in 1000, 500, 250, 125, 62.5, 31.25, 15.62µg/ml test concentration with 10µl inoculum treated bacterial cultures in well plate in triplicate and for Control group mix 100µl Tryptic soy broth without drug with 10µl Inoculum Treated bacterial cultures are incubated at 37^0 C. The bacterial test plates were observed after 48hours and O.D @ 590 nm is measured in Tecan plate reader. Determine MIC as Minimum concentration of drug giving 50% inhibition of OD as compared with control.

RESULTS AND DISCUSSION

Both samples of *Kantakari Arka* have not shown expected antibacterial activity against all selected organisms at the dose of 20µl/well. Methanolic extract of *Kantakari* showed inhibitory action against S. aureus and E. coli. at the dose of 2000µg/well. Mean Zone of Inhibition diameter with Methanolic extract of *Kantakari* against *S. aureus* was 11mm and 9mm against *E.coli.* Minimum Inhibitory Concentration of Methanolic extract of *Kantakari* against *S. aureus* was 250µg and against *E. coli* 1000µg.
The study reveals that, both samples of *Kantakari Arka* failled to exhibit the antibacterial activity.

The reason could be inferred in the following way:

Pharmaceutical perspective:

Methanol has higher penetration capacity as compared to water. Even though water and methanol are polar solvents, methanol can partially extract Non-polar (fat) molecules where it is not possible in water. Compounds like water soluble pigments and flavonoids which have higher vaporisation point will remain behind and will not come into *Arka*. The boiling point of water (100^0 C) is higher than the methanol (64^0 C), chances of thermolability of some of the active principles is more in water extracts-*Arka.* Most of the Terpenoids which are having an-

timicrobial activity are insoluble in water but soluble in ethanol, methanol, chloroform, and diethyl ether[10]. With all these probabilities it could be the inferred that, the active principles would have been extracted in higher concentrations with methanol than of water.

Pharmacological perspective:

The drugs that enter the human body initially tend to stimulate one or the other functional proteins like Receptors, Ion channels, Enzymes and Transporter proteins. Then initiate a particular cell response and thereby results in disease response.

Whereas in In-vitro, the studies are performed with microorganisms, cells, or biological molecules outside their normal biological context. Hence there is no contact of drug with biological functional proteins like Receptors, Ligands, Enzymes and Proteins. This could be the reason, for non-exhibition of expected results by drug.

Therefore, results obtained from In vitro experimental studies cannot be usually transposed, as it is, to predict the action of that drug.

CONCLUSION

Though *Kantakari Arka* decline in expected results experimentally, is mentioned to be a successful drug in classics. The experimental study results could be because of following reasons

a) As proportion of dilution,

b) Non-compatibility of drug extract,

c) Variable pharmacokinetics and pharmacodynamics of humans and microbes.

To conclude the present experimental study results, a systematic and scientific clinical study is essential.

Table1: Result of Antibacterial activity of sample 1

Test Compounds	Concentration (µg/well)	Zone of inhibition(mm)				Well reference number
		H. influenza	*E. coli*	*P. aeruginosa*	*S. aureus*	
Control	Water				-	C
Ciprofloxacin (standard)	2.5	18±0.0	17±0.0	16±0.0	15±0.0	S
K A1	20µl	-	-	-	-	1

Test Compounds	Concentration (µg/well)	Zone of inhibition(mm)				Well reference number
		S. pneumoniae	*K. pneumoniae*	*M. pneumoniae*	*L. pneumoniae*	
Control	Water					C
Ciprofloxacin (standard)	2.5	17±0.0	16±0.0	19±0.0	16±0.0	S
K A1	20µl	-	-	-	-	1

Table 2: Inhibitory activity of *Kantakari Arka* sample 2

Test Compounds	**Concentration (µg/well)**	**Zone of inhibition(mm)**				**Well reference number**
		H. influenza	***E. coli***	***P. aeruginosa***	***S. aureus***	
Control	Water				-	**C**
Ciprofloxacin (standard)	2.5	18±0.0	17±0.0	15±0.0	16±0.0	S
K A 2	20µl	-	-	-	-	2

Test Compounds	Concentration (µg/well)	Zone of inhibition(mm)				Well reference number
		S. pneumoniae	*K. pneumoniae*	*M. pneumoniae*	*L. pneumoniae*	
Control	Water					**C**
Ciprofloxacin (standard)	2.5	19±0.0	17±0.0	18±0.0	16±0.0	S
K A 2	20µl	-	-	-	-	2

Table 3: Inhibitory activity Methanolic extraction of *Kantakari*

Test Compounds	Concentration (µg/well)	Zone of inhibition(mm)		Well reference number
		Escherichia coli	*Staphylococcus aureus*	
Control	Methanol	-	-	**C**
Ciprofloxacin (standard)	2.5	20±0.0	24±0.0	S
M E K	2000	9±0.0	11±0.0	1

Table 4: Minimal inhibitory activity of Methanolic extraction of *Kantakari*

No.	Sample Code	MIC (µg/ml)		Test parameters
		S.aureus	*E.coli*	
1	Ciprofloxacin	0.25	0.5	**Methodology** Microbroth dilution technique using Culture Medium: Tryptone broth for *E.coli* and *S.auerus*. Sample test concentrations 1000, 500, 250, 125, 62.5, 31.25, 15.62 µg/ml.
2	M E K	250	1000	

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