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ERANDA PATRA (Ricinus communis L.) AS A *KRIMIGHNA DRAVYA* (ANTIMICROBIAL DRUG) WITH SPECIAL REFERENCE TO ESCHERICHIA COLI BACTERIA- AN IN VITRO STUDY

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ABSTRACT

***Background**: The recognition of any of the Dravya is through its Karma (Action). The karma is mainly explained with the help of Rasa panchaka in Ayurvedic science. Krimighna karma is one such karma which is responsible for the destruction of the krimi.*

***Methods and materials**: Swarasa, Aqueous- Methanolic and the chloroform extracts of Eranda Patra (Ricinus communis L.) was evaluated for its antimicrobial activity against Escherichia coli bacteria using Agar well diffusion method. Escherichia coli is a gram-negative bacterium residing in the colon which on pathological conditions will cause urinary tract infection, diarrhea, sepsis. As Eranda patra mainly acts on the urinary and digestive system and E. coli also affects the same system, the present work was undertaken to evaluate the Krimighna karma of Eranda patra against Escherichia coli.*

***Observation and result**: The zone of inhibition was observed using agar well diffusion antimicrobial screening method against Escherichia coli bacteria. Swarasa had shown maximum Zone of inhibition (12.66mm) for Escherichia coli bacteria when dispensed directly and moderate zone of inhibition (8mm) for swarasa with diluted concentrations after 48 hours of incubation period when compared to control group but not as that of standard group. Methanolic and the chloroform extract had shown moderate (5.5mm and 6mm) ZOI against Escherichia coli bacteria when compared to control but not as that of standard drug. The aqueous extract did not show any inhibition against the bacteria. These findings established the potential of swarasa and the extracts of Eranda patra as an effective antimicrobial agent against selected organisms. However, further studies are needed to evaluate active compounds and probable medicinal benefits in humans by clinical trials.*

KEY WORDS- Eranda, Krimighna, Swarasa, Escherichia coli, Zone of inhibition, Antimicrobial

INTRODUCTION

Traditional system of medicine mainly depends upon the plant, animal and mineral crude drugs. Plant origin drugs are mainly used in clinical practice. *Eranda* is a widely available plant and commonly used in *vatavyadhis*[1]. *Krimighna karma* (Antimicrobial activity) is mainly mentioned for its leaf in Bhavaprakasha and Kaiyadeva Nighantu[2,3]. *Eranda* is a soft-wooded small tree widespread throughout tropic and warm temperature regions of the world[4]. There are some indirect references in Vedas for microbes causing infection and infectious diseases in the name of *krimi* and *krimi rogas* which are the causative factors of a number of diseases. The word *krimi* is a broad term which includes all types of worms and microbes. The world *krimi* is derived from the root word '*Kramana'* which means attacking, overcoming, suppressing[5]. *Krimi* are said as minute and are of different shapes and colour. Some of the *krimi* are visible and some of them are invisible. Thus, they can be considered as macro and micro forms of organism. Escherichia coli is a gram-negative bacterium which is rod-shaped bacterium. It commonly resides in the intestines and which on pathological conditions will causes urinary tract infection, diarrhea, sepsis. 90% of UTI are due to Escherichia coli bacteria[6].

METHOD AND MATERIALS

The study was conducted in the following phases.

a. Pharmacognostical study

b. In vitro antimicrobial study

a. Pharmacognostical study: The leaf of *Eranda* was collected from natural habitat of Hassan and authentified by the department of Dravyaguna, SDM College of Ayurveda and Hospital, Hassan. The sample leaf powder was subjected for macroscopic-microscopic evaluation, physicochemical evaluation, phytochemical evaluation and HPTLC.

b. In vitro antimicrobial study: The study was undertaken for the following test samples.

a. *Eranda patra swarasa*

b. Aqueous extract of *Eranda patra*

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c. Methanolic extract of *Eranda patra*
d. Chloroform extract of *Eranda patra*
Method of sample preparation- The *swarasa* of *Eranda patra* and the extracts were prepared using the standard method. Agar well diffusion method was selected for conducting the invitro antimicrobial study.

PREPARATION OF NUTRIENT AGAR MEDIA

Requirements

1.Beef extract (1 g)
2.Yeast extract (2 g)
3.Peptone (5 g)
4.Sodium Chloride (5 g)
5.Distilled water -1000 ml
6.Agar -15g

Method of Preparation

The first 4 ingredients are weighed using digital balance. These extracts were dissolved in 990 ml of distilled water. The pH was adjusted to 7.2 and the volume was made up to 1000 ml. Finally, 15g agar was added to the media and autoclaved at 121˚C for 20 minutes.

Preparation of the Inoculum

Escherichia coli (MTCC 42) was procured from Microbial Type Culture Collection and Gene Bank (MTCC), IMTECH, Chandigarh. They were supplied in frozen form in sealed glass vials.

Optimum care was taken in opening the vials. The work place was cleaned in laminar air flow using 70% ethyl alcohol and UV for 20 minutes. Loopful of 48h old culture of Escherichia coli from the slants was transferred to 5 ml of sterile saline and mixed well to prepare a homogenous inoculum.

Preparation of Agar Plates

Purpose of preparing the Agar plate is to provide a larger surface area for the growth of micro-organisms. The autoclaved sterile petri plates were taken and labelled with the name of the organism and the drug along with the date of preparation. Then, the different concentrations of the drug were also labelled on the backside of the culture plates.

Table 1: Dilutions of standard and extracts

Test sample	Dilution
1mg ampicillin	600 µL
10mg aqueous extract	1000 µL
20mg of methanolic extract	1000 µL
20 mg of chloroform extract	1000 µL

Well Diffusion Method

The nutrient agar media was cooled to around 45-55˚C, around 20 ml of the media was poured into each sterile Petri plate. One ml of the inoculum was immediately added to the plate, swirled for uniform distribution. The air bubbles were removed using heat from the Bunsen burner. A sterile environment was achieved by placing two Bunsen burners. The plates were left undisturbed, cooled down and solidified to look opaque and this media is autoclaved at 121˚C for 20 minutes. Once it solidifies, equidistant wells were bored using a sterile borer.

Table 2: Different concentrations of the test sample, control and the standard group

	Swarasa	Methanolic Extract	Chloroform Extract	Aqueous Extract
Test drug	50 µL	25 µL	25 µL	10 µL
	100 µL	50 µL	50 µL	20 µL
	150 µL	100 µL	100 µL	50 µL
				100 µL
				150 µL
Control	-	50 µL	50 µL	50 µL
Standard	50 µL	20 µL	20 µL	30 µL

In the above said concentrations of test sample, standard drug and the control were dispensed into the bored well. These culture plates were incubated overnight at 37˚C and observed after 48 hours. Then, it was taken out and observed for the zone of inhibition.

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Zone of inhibition (The diameter) was measured for each concentration using a scale and noted for each concentration of *swarasa*, aqueous, methanolic and chloroform extracts respectively. The difference in the zone of inhibition between the control and the test groups were calculated for each concentration and was noted. Average of ZOI of each control group and the test drug group was calculated and compared to the standard group.

OBSERVATIONS AND RESULTS

Pharmacognostical study-

The macroscopic features of *Eranda patra (Ricinus communis* L.) was found with the following features.

Table 3: Organoleptic Characteristics

	Eranda Patra	**Powder of the *Eranda patra***
Appearance	Palmately lobed, simple with elongated petiole	-
Texture	Smooth	Smooth
Colour	Light Green in colour	Green in colour
Taste	*Tikta*	*Tikta*
Odour	Characteristic odour	Characteristic odour

Microscopic Evaluation

Eranda patra **(***Ricinus communis* L.)- Transverse section of midrib of the leaf shows the following structures- A layer of upper and lower epidermis embedded with stomata and covered with thick cuticle. The whole ridge of upper epidermis is occupied with many rows of collenchymatous tissues and shows laterally placed laminar extensions on its either side.

The lamina shows a narrow band of palisade layer underneath the upper epidermis, the remaining mesophyll tissue being occupied by many rows of spongy parenchyma.

The ground tissues of the midrib consist of radially arranged xylem vessels and narrow band of phloem embedded with plenty of clusters and few rosette crystals of calcium oxalate.

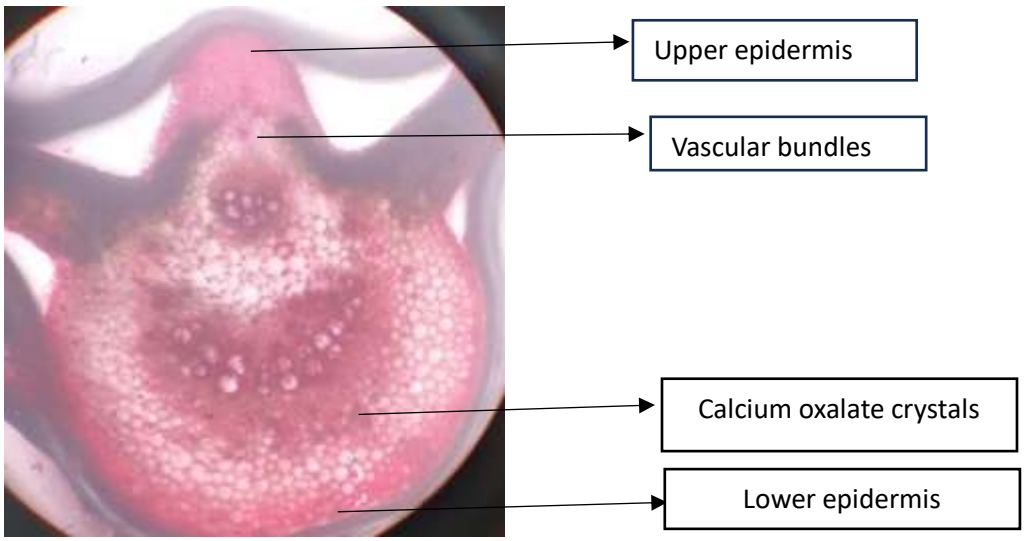

Fig.1-Microscopy of TS of *Eranda* leaf

Observations During Zone Of Inhibition

Zone of inhibition of *Eranda patra (Ricinus communis* L.*) swarasa,* aqueous extract, methanolic extract and the chloroform extract at different dosage forms were observed during the study and were recorded in tabular format from which graphs were designed.

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Table 4: ZOI of *Swarasa* of *Eranda patra (Ricinus communis* L.*)* against E. coli

	Dilution	Volume	Zone of	inhibition (mm)
Swarasa of *Eranda patra*	10+90µL	100µL	6	7
	25+75µL	100µL	7	8
	50µL+50µL	100µL	8	9
	75µL+25µL	100µL	9	10
	-	50µL	11	12
	-	100µL	12	13
	-	150µL	14	14
Control (Distilled water)	-	150µL	0	0
Standard (Ampicillin)		50µL	24	24

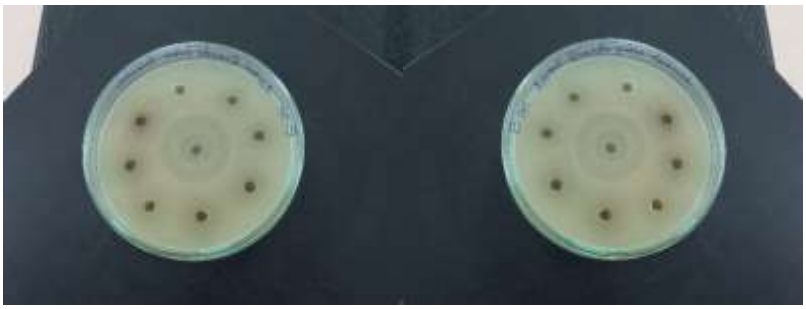

Fig. 2- Agar well plate with the test sample, control and the standard drug

Table 5: ZOI of Methanol and Chloroform extracts of *Eranda patra (Ricinus communis* L.*)* against *E. coli*.

	Volume	Zone of inhibition – (Radius in mm)	
Methanol extract of *Eranda patra* (*Ricinus communis* Linn.)	25 µl	5	5
	50 µl	5	6
	100 µl	6	7
Methanol control	50 µl	4	5
Eranda patra (Ricinus communis Linn.) powder of Chloroform extract	25 µl	5	6
	50 µl	6	6
	100 µl	6	7
Chloroform control	50 µl	5	5
Standard (Ampicillin) 1 mg/ml	20 µl	20	20

Table 6: ZOI of aqueous extract of *Eranda patra* (*Ricinus communis* Linn.) against *E. coli*

Sample	Volume	Zone of inhibition – (Radius in mm)	
Aqueous extract of *Eranda patra* (*Ricinus communis* Linn.) powder (10 mg / ml)	10 µl	0	0
	20 µl	0	0
	50 µl	0	0
	100 µl	0	0
	150 µl	0	0
Control (DD water)	50 µl	0	0
Standard (*Ampicillin*) 1mg / 600 µl	30 µl	21	21

Statistics

Arithmetic mean is applied for statistical analysis of this study.

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Table 5: Average of ZOI of each test samples, control and the standard drugs

Type of the test sample	Volume	Control ZOI (mm)	Average ZOI (mm)	Difference with the control
Swarasa of *Eranda Patra* (*Ricinus communis* L.) with dilutions		0	6.5 (6+7/2)	6.5
		0	7.5 (7+8/2)	7.5
		0	8.5(8+9/2)	8.5
		0	9.5(9+10/2)	9.5
Direct *swarasa*-	50 µL	-	11.5(11+12/2)	11.5
	100 µL	-	12.5(12+13/2)	12.5
	150 µL	-	14(14+14/2)	14
Methanolic extract of *Eranda patra* (*Ricinus communis* L.)	25 µL	4.5	4.5 (4+5/2)	0
	50 µL	4.5	5.5 (11/2)	1
	100 µL	4.5	6.5 (13/2)	2
Chloroform extract of *Eranda patra* (*Ricinus communis* L.)	25 µL	5	5.5 (11/2)	0.5
	50 µL	5	6 (12/2)	1
	100 µL	5	6.5(13/2)	1.5

Table 6: Average of ZOI of *Swarasa* of *Eranda patra*

Test Sample	Average
Swarasa (Direct)	12.66mm
Swarasa with dilutions	8mm
Control (Distilled water)	0
Standard (Ampicillin)	24 mm

The average ZOI of *Eranda patra swarasa* group was more than the control group but not as that of the standard group.

Table 7: Average of ZOI of methanolic extract of *Eranda patra*

Test sample	Average
Methanolic extract of *Eranda patra*	5.5 mm
Control (Methanol)	4.5mm
Standard (Ampicillin)	20 mm

The average ZOI of methanolic extract of the *Eranda patra* (*Ricinus communis* L.) was more than the control group but not as that of the standard ampicillin.

Table 8: Average of chloroform extract of *Eranda patra*

Test sample	Average
Chloroform extract of *Eranda patra*	6 mm
Control (Chloroform)	5 mm
Standard (Ampicillin)	20 mm

The average ZOI of chloroform extract *Eranda patra* (*Ricinus communis* L.) was more than the control group.

For Aqueous Extract

As there was no zone of inhibition found in different concentrations, average cannot be applied.

Results

a. The *swarasa* of *Eranda Patra* (*Ricinus communis* L.) with and without dilutions had shown positive antimicrobial effect against Escherichia coli bacteria in comparison with the control group but not as much as the standard group.

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b. The methanolic extract and the chloroform extracts of *Eranda patra (Ricinus communis* L.*)* has shown positive antimicrobial activity in comparison with the control group but not as that of standard drug.
c. The aqueous extract of *Eranda patra (Ricinus communis* L.) didn't show any antimicrobial activity on Escherichia coli bacteria.
By all the above observations, we can analyze that the *swarasa* of *Eranda patra* (*Ricinus communis* L.) without dilution had shown maximum antimicrobial effect when compared to different diluted concentrations of Methanolic and Chloroform extract of *Eranda patra.* Aqueous extract of *Eranda patra* didn't show any antimicrobial effect against the strain of Escherichia coli bacteria.

DISCUSSION

Based on the Selection of the Drug

The plant *Eranda* is a drug that is abundantly available and *Krimighna karma* is specifically mentioned for its leaf in Bhavaprakasha and Kaiyadeva Nighantu[2,3]. The leaf is selected because it is the part which can be easily collected and used. Rutin is the main phytoconstituent present in the leaf of Ricinus. It has been extensively studied for antimicrobial activity against various strains of bacteria[7]. It has demonstrated a profound degree of inhibition on growth of bacteria Escherichia coli. A study shows that the methanolic, ethanolic and aqueous extracts of *R. communis* leaves exhibited antimicrobial activity against four isolates of bacteria S. aureus, B. subtilis, P. aeruginosa and K. pneumoniae[8].

Based on the Selection of the form of the Drug

As the *swarasa* is considered to be the most potent *Kalpana* among *Panchavidha Kashaya Kalpana*[9] and it is easy to prepare from the leaves of the plant, this form was selected for the study. Aqueous extract is considered to be the one which is a bit similar to *Kashaya Kalpana* and it dissolves the water-soluble components of the drug. Rutin, the main constituent of the leaf of *Ricinus communis* L. is known to have high solubility in polar solvents such as methanol, ethanol, etc.[10] Hence, the methanolic extract of the leaf was selected for the antimicrobial study. Ricinine is an alkaloid present in the leaf of Ricinus. As this alkaloid is soluble in chloroform, it was selected for study.

Pharmacognostical Evaluation- All the pharmacognostical parameters indicated the genuinity of the drug.

In vitro Antimicrobial Study- Agar well diffusion method is widely used to evaluate the antimicrobial activity of plant extracts. This method is simple, qualitative and easy to perform. Hence, this method was selected to evaluate the antimicrobial activity of *Eranda patra* (*Ricinus communis* L.).

In this study, it was observed that, as the volume of the concentrations of the *swarasa* or the extract were increased, there was a subsequent increase in the zone of inhibition also. As the concentration decreases, the active molecule content has also decreased which might not be capable of destroying the strains of bacteria. There was no activity observed for aqueous extract with reference to the control as rutin is found to be slightly soluble or insoluble in water.

Based on *Rasa panchaka*[11,12,13]

Rasa- Tikta rasa. *Tikta rasa* is '*Krimin jayeth'*, which means to destroy or to kill the *krimis*. It can be taken as, by virtue of its *tikta rasa*, it helps in destroying the *krimis* or helps in stopping the growth of the organisms.

Guna - Laghu, Ruksha. Laghu guna - It has *Kaphahara* property which also hinders the growth of the *Krimi*. *Ruksha guna* - The main action of *Ruksha guna* is *Shoshana, Sthambhana* and also *kaphahara* which helps in drying or shrinks the size of the cells. Thus, it will act as *Krimi shoshaka* and it stops the growth of the bacteria.

Veerya - Ushna veerya. Ushna veerya of the *dravya* will cause *dhatu kshaya* which helps in destruction of the *Krimi* which may help the organism in preventing its multiplication or further growth. This *guna* also causes destruction of the cell components of the bacteria because of its *pachana* property. Thus, it helps in both bacteriostatic and bactericidal action.

Doshaghnata- Vatakaphahara

Growth of the *Krimi* is mainly due to *Vata* and *Kapha dosha*. *Eranda patra* having *Kapha* and *vatahara* property which inhibits the growth of *Krimi.*

Based on the Phytoconstituents

Eranda patra (*Ricinus communis* L.) is having Ricinine (1%), Quercetin 3-O-B- rutinoside (rutin) in it[7]. And the preliminary phytochemical analysis determines the presence of tannins, tannic acid, proteins and carbohydrates in it. As the phytochemical constituent rutin is slightly or insoluble in water, the antimicrobial activity may not have been found in the aqueous extract. And it is highly soluble in polar solvents such as methanol (55 g/L), ethanol (5.5 g/L), etc., the antimicrobial effect was found in methanolic extract of *Eranda* (*Ricinus communis* L.). Tannins hamper the plasma coagulating the property of Escherichia coli, preventing the

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formation of fibrin rich membranous structures by the organisms[14]. Tannins have shown antimicrobial effects against various microorganisms. Rutin inhibit the synthesis of DNA, RNA and related macromolecules in the bacteria and hence prevent the growth of the bacteria[15].

CONCLUSION

Eranda patra (*Ricinus communis* L.) *swarasa*, methanolic and the chloroform extracts possess antimicrobial activity (*Krimighna karma*) against the Escherichia coli bacteria but not as that of standard drug.

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