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Pharmaceutical Study of Ardraka Khand and Its Bactericidal Effect



1*Palak Chaudhry, 2Neha Arya

¹M.D Scholar, P.G Department of Rasa Shastra and Bhaishajya Kalpana, Patanjali Bhartiya Ayurvigyan evam Anusandhan Sansthan, Haridwar, Uttarakhand, India.

²Associate Professor, P.G Department of Rasa Shastra and Bhaishajya Kalpana, Patanjali Bhartiya Ayurvigyan evam Anusandhan Sansthan, Haridwar, Uttarakhand, India.

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ABSTRACT

Ardraka Khand mentioned in Bhava Prakash is a drug of choice in Udarda, Shitapitta and Kotha, Kasa, Swasa etc. Khand preparations are easy to administer and lasts long due to presence of sugar in them. In-vitro Antibacterial study of this formulation is not established yet, so the present study was planned subsequent to the Pharmaceutical study, which is the first step in a research process of any formulation. Ardraka Khand was prepared by adopting the reference of Bhava Prakash and a constant heat was maintained during the whole procedure. The antibacterial Study of Ardraka Khand was evaluated against the bacteria E.coli, Staphylococcus aureus and Salmonella. Bacteria E.coli has shown mild activity, Staphylococcus aureus has shown resistance and Salmonella has shown Intermediate sensitivity against 500mcg potency of Ardraka Khand.

INTRODUCTION

Rasa Shastra means "Science of Mercury". It is a branch of Ayurveda which deals with metals and minerals to produce drugs with higher efficacy and in low doses. Bhaishajya Kalpanais the branch which deals with the preparation of medicines such as tablets, powders, decoctions etc. It not only deals with the preparation of medicines but it also includes Quality control measures right from collection of raw materials up to finished product.

Skin is the largest organ of the body. Ayurvedic texts described five *Gyan indriyas and twak* (Skin) is one of them and it is responsible for "*Sparsha Gyan*" (touch sensation). Skin diseases are a major health problem and always a great concern to mankind. In *Ayurveda*, all skin diseases go under the classification of *Kustha and Ksudra rogas. Kustha* one among the *Ashtmahagada*, *Santarpanjanaya and Raktpradoshaja vikara*. Seven materials affected morbidly are the causative source of Kustha such as *Tri doshas- Vata, Pitta, Kapha —* vitiated by etiological factors and four *dushya sharir dhatus — twak, mamsa, rakta and lasika —* affected with affliction by doshas. The seven materials in this way are causative factors of seven types of *Kustha*. Arising from this source they afflict the whole body. [1]

Since the times immemorial the *Kustha* is the most baneful disease afflicting the human beings. Thus, it is necessary to deal with this Kustha roga considering all factors like *dosha*, *desha*, *kala*, *bala*etc.

Ayurveda classics described various types of formulations and *Khand kalpana* is one of them. The root meaning of the word *Khand* means a fragment, a part or a piece. Khand preparations are same as that of *Avaleha*.^[2] It has thesame essential ingredients and specified ratio. If the preparation of *Avaleha* is boiled one step ahead and brought into granular or powder form then it is called *Khand*. Its assimilation starts in the buccal cavity. It can be given to all age groups for their palatability and easy method of administration. Another advantage is that it has a long shelf life and good taste.

Ardraka Khand, mentioned in Bhava Prakash is a herbal formulation, that consists of Ardraka, Pippali, Pippalimula, Maricha, Shunthi, Mustak, Chitrak, Vidanga, Nagkesara, Twak, Ela, Tejpatra, and Kachur, Goghrita, Godugdha and Sharkara.^[3]

Ardraka Khand is indicated in the disease *Shitapitta* (Urticaria), *Kasa, Swasa, Rajyakshma, Gulma* and acts as *Agnideepaka and Rasayana*. [4] Additionally, most of the individual drugs of this formulation show *Krimihara, Kusthahara, Kanduhara, Dipaniya and Pachniya*

properties which are similar toantihelminthic (destruction of intestinal parasites), antihistaminic (helps in relieving allergic reactions), anti-inflammatory, carminative and digestive properties.

Keeping all these factors in mind there is a need of hour to find out the bactericidal effect of *Ardraka Khand* as indicated in classical texts.

AIMS AND OBJECTIVES

- 1. To prepare Ardraka Khand.
- 2. To evaluate the Antibacterial activity of *Ardraka Khand*.

MATERIALS AND METHODS

It can be studied under two steps: 1) Pharmaceutical Study and 2) Antibacterial Study

1) Pharmaceutical Study

- * Collection of raw drugs: Raw materials were collected and procured from Govindpuri, Haridwar. *Godugdha, Go Ghrita and Sharkara* were procured from Patanjali Mega Store, Phase 1, Haridwar. *Ardraka* was procured from the local market of Roorkee.
- * Authentication of herbal raw drugs: Raw materials were authenticated by the Department of Dravyaguna, Patanjali Bhartiya Ayurvigyan evam Anusandhan Sansthan, Haridwar.
- * Grinding and sieving of herbal raw drugs: Powders of raw drugs (Pippali, Pippalimula, Maricha, Shunthi, Mustak, Chitrak, Vidanga, Nagkesara, Twak, Ela, Tejpatra, and Kachur) were prepared with the help of mortar and pestle and the sieve no. 80.
- * **Preparation of Ardraka Khand:** The preparation of *Ardraka Khand* was done in PG department of Rasa Shastra and Bhaishajya Kalpana, Patanjali Bhartiya Ayurvigyan Evam Anusandhan Sansthan, Haridwar.

The composition of ingredients of *Ardraka Khand* is mentioned in table 1:

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Table 1: Composition of ingredients of Ardraka Khand

S. No.	Ingredients	The quantity given as per reference of Bhava Prakash	Quantity taken
1.	Ardraka	1 Prastha (768 gms)	96 gms
2.	Go ghrita	2 Kudava (384 gms)	48 gms
3.	Go dugdha	2 Prastha (1536 gms)	192 gms
4.	Sharkara	1 Prastha (768 gms)	96 gms
5.	Pippali	1 Pala (48 gms)	6 gms
6.	Pippalimula	1 Pala (48 gms)	6 gms
7.	Maricha	1 Pala (48 gms)	6 gms
8.	Shunthi	1 Pala (48 gms)	6 gms
9.	Chitrak	1 Pala (48 gms)	6 gms
10.	Vidanga	1 Pala (48 gms)	6 gms
11.	Mustak	1 Pala (48 gms)	6 gms
12.	Nagkeshar	1 Pala (48 gms)	6 gms
13.	Twak	1 Pala (48 gms)	6 gms
14.	Ela	1 Pala (48 gms)	6 gms
15.	Patra	1 Pala (48 gms)	6 gms
16.	Kachura	1 Pala (48 gms)	6 gms

Ardraka Khand was prepared according to the reference of Bhava Prakash(Bh. Pr. 55/16-21). The required quantity of the powdered raw drugs, Ardraka, Godugdha, Go ghrita and Sharkara were accurately weighed. Wet Ardraka (96 grams) was collected, and cleaned and the external covering was removed and made into fine kalka form.

Then, kalka was fried with *Goghrita* (48 grams) on mandagni till Ghrita was separated from the kalka. Milk (192 grams) was added to this fried *kalka* and boiled until it achieved the soft

consistency. Sugar (96 grams) was taken in another vessel, water was added and the whole mixture was boiled on *mandagni*. Fried *ardraka kalka* was added to this and mixed thoroughly. Then, afine powder of drugs 6 grams each (*Pippali, Pippalimula, Maricha, Vidanga, Chitrakmula, Musta, Shunthi, Nagkeshar, Twak, Ela, Patra and Kachur*) were added to the mixture, mixed thoroughly and was made into a homogenous mixture. The mixture was allowed to cool for some time and it was preserved in a glass vessel.

Preparation of Ardraka Khand is shown in the images below:



Figure 1: Raw Materials

Figure 2: Pounding of Raw Material



Figure 3: Sieving of raw material



Figure 4: Powdered raw material



Figure 5: Raw Ingredients



Figure 6: Frying of ArdrakaKalka in ghee

Figure 7: Addition of milk



Figure 8: Paste-like consistency

Figure 9: Preparation of sugar syrup



Figure 10: Two-thread consistency



Figure 11: Addition of sugar syrup

Figure 12: Paka lakshana



Figure 13: Addition of prakshepa dravyas

Figure 14: Mixing



Figure 15: Preparation of granules

Figure 16: Prepared Ardraka Khand

Observations-

The *Adraka Khand* is brownish in colour. Typical smell of *Ardraka* appears during the process. Effervescence was observed after adding Sharkara to the water, which subsided on continuous stirring. After gradual thickening of the syrup, *lakshan-liketantumatwa* (thread like) and *Darvi Pralepa* (adhesion of the syrup to the spoon) was observed on heating. After addition of *Prakshepa dravyas*, the colour the mixture becomes darker. *Siddhi Lakshans* for the paka were observed in the preparation of granules.

Characteristics of Ardraka Khand are given in the Table 2.

Table 2: Characteristics of Ardraka Khand

S. No.	Characters	Ardraka Khand
1.	Colour	Dark Brown
2.	Odour	Aromatic
3.	Taste	Characteristic taste
4.	Texture	Rough
5.	Form	Semi solid (Granules)

2. Antibacterial Study

To evaluate the Antibacterial activity of ArdrakaKhand, thefollowing materials were used.

Materials:

A) Drugs: Ardraka Khand, Gentamicin and Ciprofloxacin

B) Micro-organisms:

Bacteria- Staphylococcus aureus, Escherichia coli, Salmonella

- **C) Solvent -**DMSO (Dimethyl sulfoxide)
- **D**) **Equipment-**Water bath, Loops and loop holder, Borer, Hot air oven, Autoclave, Incubator
- **E) Glasswares-**Distillation apparatus, Petri dish, conical flask, Test tubes, Beakers, Funnel, Stirrer, Digital balance

Methods:

- A) Preparation of solutions:
- a) **Preparation of control solution-** DMSO (Dimethyl sulfoxide) is considered as a control solution.
- **b) Preparation of test solution-** 10 mg of the sample (Ardraka Khand) is weighed and dissolved in 10 ml of the solvent (DMSO). The stock concentration is 1 mg/ml.
- c) **Preparation of standard solution-**1mg/ml concentration of Gentamicin and Ciprofloxacin was prepared, used as a standard against microorganisms.
- B) Culture media: Soyabean casein digest agar media

C) Preparation of inoculums:A loopful of organisms are emulsified in 100 ml sterile growth media under sterile conditions and incubated in an incubator at 37°c for 72 hours.

D) Preparation of Agar plates:

- 5 ml of prepared inoculums were added to 45 ml of flask containing nutrient agar at 37°c.
- Then, it was poured immediately into a sterile Petri dish to a depth of 5mm.
- The plates were allowed to solidify at room temperature for 12 hours.
- The surface of the agar layer is kept for drying before use.
- •By using sterile cavity borer, wells were made properly to enable the introduction of test solution (Ardraka Khand), standard drugs Gentamicin and Ciprofloxacin, and blank as a control (DMSO) precisely with the help of micropipette.
- After 30 minutes Agar plates were incubated at 37°c for 72 hours.
- •After 72 hours, the zone of inhibition was examined and measured using the zone of inhibition scale.

555

RESULTS

1) Results of Pharmaceutical Study:

It consists of grinding and sieving of herbal raw drugs and preparation of Ardraka Khand.

The results of grinding and sieving of herbal raw drugs are given in Table 3.

Table 3: Results of grinding and sieving of herbal raw drugs.

S.No.	Name of drug	Initial weight (in grams)	After pounding (in grams)	After grinding (in grams)	After sieving (in grams)	Loss in weight (%)
1.	Pippali	50	48	41	25	50%
2.	Pippalimula	50	49	47	17	66%
3.	Maricha	50	49	47	22	56%
4.	Shunthi	50	50	49	30	40%
5.	Chitrakmula	50	49	48	17	66%
6.	Vidanga	50	49	45	14	72%
7.	Mustak	50	48	45	17	66%
8.	Nagkeshar	50	49	47	15	70%
9.	Twak	50	49	48	15	70%
10.	Ela	50	49	48	18	64%
11.	Patra	50	49	45	13	74%
12.	Kachura	50	48	48	28	44%

Results of preparation of Ardraka Khand

- a) Final quantity of finished product- 180 Grams
- b) Total time duration- 5 hours
- c) No. of days for preparation of Adraka Khand- 2 days
- d) Form- Semi solid (Granules)

2) Results of Antibacterial Study:

In the present study, the Cup plate method was selected for the evaluation of Antibacterial Study of *Ardraka Khand*.

In vitro anti-bacterial activity tests for a sample of ArdrakaKhand against E coli, Staphylococcus aureus and Salmonella are given in the Table 4.

Table 4: Results of the Antibacterial Study

S. NO.	Antibacterial Activity against	Gentamicin	Ciprofloxacin	Ardraka Khand (500mcg)
1.	Escherichia coli (EMB Agar)	27 mm	21 mm	10 mm
2.	Staphylococcus aureus	22 mm	-	-
3.	Salmonella	32 mm	30 mm	20 mm
4.	Escherichia coli (Macconkey Agar)	27 mm	21 mm	10 mm



Fig:17 Antibacterial activity against E.coli (EMB Agar) against Staphylococcus aureus

Fig:18 Antibacterial activity



Fig:19 Antibacterial activity against Salmonella Fig:20 Antibacterial activity against E coli(Macconkey Agar)

DISCUSSION

Khand Kalpana is one of the important *Upkalpanas in BhaishajyaKalpana*. Khandpreparations are thesame as that of Avaleha. If the preparation of Avaleha is boiled one step ahead and brought into granular or powder form then it is called Khand. Since these preparations are dry in form, they have less chance of fungal attacks and also more convenient to pack, preserve and dispense these preparations. Bitter drugs can be easily consumed in this form because of the sweetening agents present in it. More shelf life is put into the preparations when compared with other liquid and semi solid preparations.

Grinding and sieving of raw drugscan be co-related with modern techniques like Size reduction which corresponds to *Mardana*, Size separation which corresponds to *Sivana* and Mixing of powders which explains the mixing of more than one drug.

The size reduction technique increases surface area and provides consistency, which enhances the rate of dissolution when the solid particle comes in contact with the gastric or intestinal juices.

The mechanism of size reduction might shift with the nature of material. When sufficient amount of stress in the form of impact, shear and compression are applied, the weak flaws appears into cracks and results in cleavage. Thus, additional surface area is obtained with smaller particles in the process of size reduction. Size reduction technique gives particles of different sizes, in order to achieve the same size the materials are subjected to separation

technique known as sieving (Also called as sifting, screening etc.) and is done with the help of Sieves. In this technique, particles of different sizes are passed through a sieve of uniform size and the particles drop down through the openings because of gravity.

Ardraka Khand was prepared according to the reference of Bhava Prakash (Bh. Pr. 55/16-21). The required quantity of the powdered raw drugs, Ardraka, Godugdha, Go ghrita and Sharkara were accurately weighed. Wet Ardraka was collected, cleaned and the external covering was removed and made into fine kalka form, followed by frying with Goghrita on mandagni till Ghrita was separated from the kalka. The average yield of Kalka after frying with Goghrita was found to be 60%, which indicates complete evaporation of moisture content from the Ardraka Kalka. During frying the average temperature was maintained between 60-70°c, due to which active constituents are not evaporated, only moisture evaporates.

Milk was added to this fried *kalka* and boiled until it achieved the consistency like a soft paste. Sugar was taken in another vessel, water was added and the whole mixture was boiled on mandagni. In this process, Sucrose is converted to glucose and fructose.

Fried *Ardraka kalka* was added to this and mixed thoroughly for the homogenous mixture. Then, fine powder of drugs (*Prakshepa dravyas*) was added to the mixture, mixed thoroughly and made into a homogenous mixture. The mixture was allowed to cool for some time and it was preserved in a glass vessel.

Average time required for the preparation of Khand was about 5 hours. Temperature during the whole process was maintained under 100°c, for optimum preservation of the chemical constituents in the final product.

Antibacterial Study is a technique in which the response of an organism can be seen against particular antibacterial agents. Various methods are used for the evaluation of antibacterial activity of a drug. In the present study, Cup plate method was selected for the evaluation of Antibacterial Study of *Ardraka Khand*.

Gram Positive (Staphylococcus aureus), Gram Negative (E.coli, Salmonella) were selected for the study. Because these organisms occur in large number and responsible for various infectious diseases like skin disorders, cold, coughs, diarrhoea etc.

Citation: Palak Chaudhry et al. Ijppr.Human, 2023; Vol. 27 (4): 546-562. 559

Each microorganism has specific growth requirements. In this present study, Soyabean casein digest agar media has been used as culture media. Agar universally has been used as solidifying agent since ages.

Test solution (Ardraka Khand), standard antibacterial drugs Gentamicin and Ciprofloxacin and blank as a control (DMSO) were used.

Results of the Antibacterial study are expressed by determining the zone of inhibition by using zone of inhibition scale.

- At the concentration of 500mcgArdrakaKhand shows 10mm zone of inhibition, Gentamicin and Ciprofloxacin shows 27mm and 21mm zone of inhibition respectively against E coli (EMB Agar).
- At the concentration of 500mcgArdrakaKhand shows no zone of inhibition, while Gentamicin shows 22mm and Ciprofloxacin shows no zone of inhibition against Staphylococcus aureus.
- At the concentration of 500mcg Ardraka Khand shows 20mm zone of inhibition, Gentamicin and Ciprofloxacin showa 32mm and 30mm zone of inhibition respectively against Salmonella.
- At the concentration of 500mcg Ardraka Khand shows 10mm zone of inhibition, Gentamicin and Ciprofloxacin showa 27mm and 21mm zone of inhibition respectively against E coli (Macconkey Agar).

It is clear from the results that Ardraka Khand has shown good results against Gram-negativebacteria(E.coli, Salmonella) rather than Gram-positive bacteria (Staphylococcus aureus).

CONCLUSION:

Khand Kalpana increases the shelf life of the formulation. Bitter drugs can be easily consumed in this form because of the sweetening agents present in it. *Adraka Khand* was brown in colour, with aromatic odour, characteristic taste and rough texture.

Bacteria E.coli (EMB Agar) and E. coli (Macconkey Agar) have shown mild activity against 500mcg potency of Ardraka Khand. Bacteria Staphylococcus aureus has shown resistance against 500 mcg potency of Ardraka Khand. Bacteria Salmonella has shown Intermediate sensitivity against the 500 mcg potency of Ardraka Khand. So, it can be concluded that Ardraka Khand has shown good results against the bacteria Salmonella.

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	Author Name – Corresponding Author- Dr. Palak
	Chaudhry
Imaga	Author Affiliation- M.D Scholar, P.G Department of
Image Author -1	Rasa Shastra and Bhaishajya Kalpana.
Author -1	Author Address/Institute Address – Patanjali Bhartiya
	Ayurvigyan evam Anusandhan Sansthan, Haridwar,
	Uttarakhand, India.
	Author Name- Dr. Neha Arya
	Author Affiliation- Associate Professor, P.G
Image	Department of Rasa Shastra and Bhaishajya Kalpana.
Author -2	Author Address/Institute Address- Patanjali Bhartiya
	Ayurvigyan evam Anusandhan Sansthan, Haridwar,
	Uttarakhand, India.
	Author Name
Image	Author Affiliation
Author -3	Author Address/Institute Address
	Author Name
Image	Author Affiliation
Author -4	Author Address/Institute Address
_	Author Name
Image	Author Affiliation
Author -5	Author Address/Institute Address