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RESEARCH ARTICLE

INVITRO ANALYSIS OF AYURVEDA DECOCTION AND LIPID FORMULATION PREPARED FROM KRIMIGHNA MAHAKASHAYA GANA AGAINST DERMATOPHYTES.

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Abstract

Many classical literatures are mentioned in *Ayurveda* and *Charaka samhita* is one among them.*Charaka Samhita* has mentioned 50 groups of drugs as *Mahakashaya gana*. *Krimighna Mahakashaya gana* is one among it which contains 10 drugs used against macrobes and microbes. However the dosage form in which this group of drugs has to be used is not clear. The liberty of deciding the suitable dosage form is left to the descretion of the physician.

Kwatha(Decoction) is a primary dosage form where the drugs are boiled with prescribed amount of water and reduced to specific amount. In this preparation water soluble active principles present in the drugs are extracted. When the *Kwatha* is processed with the drugs in *Krimighna Mahakashayagana* the attributes of the drugs will be transferred in to water media.

Taila(oil) are preparations in which oil is boiled with prescribed *Kwatha*(decoction) and *Kalka(*paste) of drugs according to the formulae for specific duration of time, fat soluble active principles of herbs will be transformed to oil media, which can be used internally as well as externally as specified . When the *Taila*(oil) is processed with the drugs in *Krimighna Mahakashaya gana* the attributes of the drugs will be transferred in to oil media.

Krimi mentioned in *Ayurveda* can be compared with microorganism and macroorganism. Among microorganisms, study of pathogenic Fungihas received only scant attention in comparison with other Pathogens. With the control of most Bacterial infections in the developed countries, fungal infection has assumed greater importance.

Though anti microbial activity of some of the single constituent of above mentioned *Krimighna Mahakashaya gana*(Combination of 10 drugs) has been carried out, study of the whole group has not carried out. So to establish the *Krimighna* action w.s.r to anti fungal activity of the drugs of this group is taken up. Since the dosage form in which the medicine has to be administered is not clearly mentioned, two dosage forms namely *Kwatha*(decoction) and *Taila*(oil) are selected for present study.

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Introduction:-

Branch of *Ayurveda* which deals with the preparation of medicine is called *Bhaishajya Kalpana*.*Bhaishajya* means the medicine which treats diseases or controls it without any side effects[1]. *Kalpana* means the method by which various formulations are prepared and such formulation is also known as "*Kalpa*",the form attained will be suitable for body which enhances the health of the body[2].

The role of *Bhaishajya Kalpana* in potentiating the drug action has been explained in *Ayurveda*. As per *Ayurveda PanchaVidha Kashaya Kalpana* like *Swarasa*(juice extract from herbal drugs), *Kalka*(drug paste), *Kashaya*(decoction), *Hima*(cold infusion) and *Phanta*(hot infusion) are considered as primary *Kalpana* and the base of all formulations[3]. *Kwatha*(Decoction) is a primary dosage form where the drugs are boiled with prescribed amount of water and reduced to specific amount. In this preparation water soluble active principles present in the drugs are extracted. *Sneha Kalpana* is a secondary *Kalpana* processed using primary *Kalpana* .It is a pharmaceutical procedure adopted to produce a medicament from the substances such as *Kalka(Drug paste), Kwatha(decoction),* and *Drava Dravyas(liquid base)* in specific proportions by subjecting them to specified heating pattern and duration. By this process, there happens a transformation of active therapeutic properties of the ingredients to the solvents, and hence, which helps in recovery of fat-soluble as well as water-soluble chemical constituents[4,5].*Taila Kalpana* (oil) is one form of *Sneha Kalpana*. *Krimighna Maha Kashaya Gana*[6]is a group of ten drugs mentioned in books of *Ayurveda* against *Krimi*. It contains *Akshiva(Moringaoleifera), Maricha (Piper nigrum), Gandira(Euphorbia antiquorum), Kebuka (Costusspeciosus),Nirgundi (Vitexnegundo), Vidanga (Embeliaribes), Kinihi(Achyranthesaspera), Swadamshtra (Tribulusterestris),Vrishaparni (Ipomea variety) and Akhuparni(Ipomeareniformis).Krimi*[7] mentioned in Ayurveda can be compared with microorganism and macro organism. Among microorganisms Fungal infection is a condition faced in present era, study of pathogenicfungi[8]has received only scant attention in comparison with other Pathogens. Hence an attempt is made to prepare *KrimighnaKwatha*(decoction from combination of 10 drugs) and *Taila*(oil preparation with 10 herbal drugs) and analyze using suitable parameters. And In vitro assessment of both the formulations for antifungal effect was also carried out with selected modern parameters.

Methodology adopted

The methodology of the present work was carried under following sections.

1. Pharmaceutical study
2. Analytical study
3. Experimental study

1. Pharmaceutical study

A. Collection of the drug
B. Authentication of the raw drugs
C. Preparation of the *Krimighna Kwatha*(decoction from drugs of *Krimighna Maha KashayaGana*[6])
D. Preparation of the *Krimighna Taila*(oil preparation from *Krimighna Maha Kashaya Gana*[6])

A. Collection of the drug:

The total 10 raw drugs were collected among which 7 were dry drugs and 2 were wet drugs and instead of *Vrishaparni, Akhuparni* was taken in double quantity since *Vrishaparni* and *Akhuparni* is of same *ipomea* variety.The drugs required for the preparation of medicine were collected from Centre for Indian Medical Heritage (CIMH), Kanjikode Kerala india .

B. Authentication of the drug

The authentication of all the raw drugs was done at the Department of Dravyaguna, in Shri Dharmasthala Manjunatheshwara College of Ayurveda, Hassan.

C. Preparation of Krimighna Kwatha:

Materials:

Sieve size No 20-40, Stainless steel vessel of height-9.5cm depth 7.5cm, internal diameter-15.5cm, Potable water, external diameter-17.5cm and circumference- 45cm and thickness of 1mm, LPG stove and 10 drugs.

Methods:
Fresh Krimighna Kwatha(decoction from drugs of *Krimighna Maha KashayaGana*[6])was prepared at Shri Dharmasthala Manjunatheshwara Centre for Research in Ayurveda and Allied Sciences, Udupi Karnataka India and used for the study. Coarse powder of 7 dry drugs which passed through sieve no 20-40 was taken in quantity of 5 gm and 3 wet drugs were taken in double quantity 10Gm[9]. Processing of raw dry drugs was done at the Department of Rasashastra and Bhaishajya Kalpana, Shri Dharmasthala Manjunatheshwara College of Ayurveda Hassan. And wet drugs were crushed from Shri Dharmasthala Manjunatheshwara Centre for Research in Ayurveda and Allied Sciences, Udupi Karnataka india. The amount of water added was sixteen parts to that of total weight of all the ten drugs and it was reduced to one-eighth of the original quantity[10]. To the mixture of all drugs1040ml of potable water was added and kept for boiling in stainless steel vessel. The heating was done on LPG stove and level for reduction was checked by using a measuring scale. After the appropriate reduction of water the *Kwatha*(decoction)was filtered and used for in-vitro study.

D.Preparation of *Taila*
The preparation of *Taila*(oil) was done at the Department of Rasashastra and Bhaishajya Kalpana, Shri Dharmasthala Manjunatheshwara College of Ayurveda Hassan Karnataka. Steps adopted were:
1. Preparation of *Krimighna Kwatha*(decoction from drugs of *Krimighna Maha Kashaya Gana*[6]**)** for *Taila*(oil prepared from drugs of *Krimighna Maha Kashaya Gana*[6]**)**
2. Preparation of *Kalka* (Drug paste)
3. Preparation of *Taila*(Medicated oil)
The process is explained below:

1. Preparation of *Krimighna Kwatha*:
Materials: Sieve size No 20-40, stainless steel vessel of height 25cm, depth of 25cm, internal diameter 37.5cm and external diameter of 40cm, with thickness of 2mm having circumference of 116cm, potable water, LPG stove, 10 drugs.
Methods: The preparation of *Kwatha*(decoction) was carried out at Department of Rasashastra and Bhaishajya Kalpana, Shri Dharmasthala Manjunatheshwara College of Ayurveda Hassan Karnataka. Coarse powder of 7 dry drugs of the *Krimighna Mahakashaya*(decoction from drugs of *Krimighna Maha Kashaya Gana*[6]**)** which passed through sieve size no 20 -40, was taken in quantity of 100gm and 3 wet drug were taken in quantity of 200 gm[9].This mixture was added with 8000ml of potable water and kept for heating on LPG stove in a stainless steel vessel. The amount of water taken was eight parts of all drugs and reduced to one fourth of the total quantity[11]. Water level for reduction was checked by using a measuring scale. Then the *Kwatha*(decoction) was filtered and used for preparation of *Taila*(oil).

2. Preparation of Kalka (Paste):
Materials: Sieve size No 100-120, *Khalwayantra*(mortar and pestle) -Depth-13cm, Thickness-4cm,Width-34cm, Breadth-21cm, Height-20cm, Pestle-30cm, 10 drugs.
Methods: *Kalka* (Paste) was prepared from fine powder of 7 dry drugs and 3 wet drugs. All dry drugs were taken in quantity of 44g and coarsely powdered in *Khalwayantra*(mortar and pestle)and finely powdered in mixer grinder along with wet drugs. The particles of the fine powder were of the sieve size No 100-120. 50 ml of potable water was used for preparation of *Kalka* (Paste) into a bolus. It was weighed to 83g in wet state and used for the *Taila Paka*(oil preparation from *Krimighna* drugs).

3. Preparation of *Taila*(oil):
Materials: Stainless steel vessel of height 12cm, depth 11.5cm, internal diameter of 21.5cm and external diameter of 24.5cm with a thickness of 1mm and circumference of 64cm, LPG stove, *Kalka* of 10 drugs
Methods: The *Kwatha*(Decoction) and *Kalka*(Paste) prepared as above were used for the *Taila* Paka. 83g of Kalka (Paste), 1960ml of *Kwatha*(Decoction), and 500ml of *Taila*(oil) was used for *Taila Paka*(oil preparation from *Krimighna* drugs). The *Taila Paka*(oil preparation from *Krimighna* drugs) was done in a stainless steel vessel. LPG stove was used for the heating purpose.The mild flame was maintained during whole process. The changes in the *Taila*(oil) and *Kalka*(Paste) along with temperature changes were noted for every 15min.When the *Taila* (oil) attained *Kharapaka*[12](when the oil imbibed active principles from the drugs) flame was stopped.The total time taken for completion of the *Taila*(oil) was 5hours and 15min. After that the *Taila*(oil) was filtered and amount of the *Taila*(oil) obtained was measured it was 460 ml, it was used for analytical and in vitro study.

Analytical Study
In the present study,Analytical evaluation of *Krimighna Kashaya Churna*(Powder repared from *Krimighna MahakashayaGana*[6]), Krimighna Kwatha (Decoction pepared from *Krimighna MahakashayaGana*[6]) and Taila(oil prepared from *Krimighna MahakashayaGana*[6]) is carried out to develop preliminary standards.

Experimental Study
For validation of *Krimighna Kwatha*(decoction prepared from *Krimighna Mahakashaya Gana*[6])and *Taila*(Medicated oil) prepared from *Krimighna Mahakashaya Gana*[6]In-vitro study was done by using the *Dermatophytes* for checking its efficacy. The genera *Trichophyton, Microsporum, Candida, aspergillus* and *Epidermaphyton* are the principle etiologic agents of the *Dermatophytes*[13], *Dermatophytosis*[14] is a superficial fungal infection of keratinized tissues. The species used for present study is *Trichophyton tonsurans*[15], *Microsporum canis*[15], *Aspergillus niger*[15], *Candida albicans*[16].

Anti microbial study types[17]:
1. Diffusion test
2. Dilution test

Diffusion test:
Diffusion consists of two method i.e. Agar well diffusion, Agar disc

Diffusion
Agar well diffusion:
The Agar diffusion assay is one method for quantifying the ability of antibiotics, to inhibit microbial growth against test drug. A known quantity of micro- organism is grown on agar plate. The well is bored with help of borer, standard drug and test drug of desired concentration is poured in well. If the organism are susceptible to a particular antibiotic or a test drug , an area of clearing zone where organism are not capable of growing will be noted i.e. called a zone of inhibition. If the compound is effective against an organism at certain concentration, no colonies will grow and this is called the zone of inhibition. In general, larger zones correlate with smaller minimum inhibitory concentration (MIC) of antibiotic for that organism. Inhibition produced by the test is compared with that produced by known concentration of a reference compound.

b) Agar disc diffusion:
It is same as the previous method instead of wells, the disc are placed in agar media (both standard and test drug disc) later zone of inhibition is noted. The disc should not be placed closer than 24 mm in agar plate. Not more than 12 discs should be placed on a 150 mm plate. The disc must be pressed down with forceps to ensure complete contact with the agar surface.

2) Dilution method:
Serial dilution of the drug is prepared and inoculated with the test microbe. In the tube dilution method, serial dilutions of the drug in broth are taken in tubes and a standardized suspension of the test microbe which is inoculated. After overnight incubation, the minimum inhibitory concentration (MIC) is read by noting the lowest concentration of the drug that inhibits growth. In present study Agar diffusion method is followed to assess the samples for its activity.

Material and method:-

Materials:
A) Drugs:
Krimighna Kwatha(decoction from *Krimighna Mahakashaya Gana*), *Krimighna Taila*(Taila from *Krimighna Mahakashaya Gana*)
B) Micro- organism:
Fungal organism:
The *Dermatophytes* produce infection that involves the superficial layer of the body, including areas of the body, including the hair, skin and nails. Among them the organisms used for the study are *Trichophyton tonsurans*-MTCC CODE 8475, *Microsporum canis*-MTCC CODE-2820, *Candida albicans*-MTCC CODE 183, *Aspergillus niger*-MTCC CODE-10180

Methods: Anti microbial activity was done by Agar – well diffusion method. Assessment through disc diffusion study was measured by following zones:
1) Sensitive zone
2) Intermediate Zone

Agar well diffusion method
Principle –
1. Diffusion of the drug from a cavity through the solidified agar layer of a petridish
2. Prevention of the growth of the added micro organisms in a circular area or zone around the cavity containing a solution of the drug
3. Place was cleaned in laminar air flow by using 70% ethyl alcohol and UV light was switched on for 20 minutes.
4. 15 ml of the Sabouraud's agar media ,yeast extract dextrose media was poured uniformly over the sterilized petridish.
5. 1 ml of Sabouraud's broth, yeast extract dextrose media containing the organism was added uniformly over petridish, mix well and allow the media to solidify
6. Six equidistant wells on the plate were made
7. Test were conducted for different volume of sample (10, 25, 50, 100 and 150 µl) separately.
8. Seperately take 100 micro litre of Amphoterecin b ,and fluconazole as standard
9. Incubation of all the petridishes .
10. Microsporum canis – Incubation was done for 10 days
11. Trichophyton tonsurans - Incubation was done for 7 days at 25^0c
12. Candida albicans- Incubation was done for 48 hours at 30^0c
13. Aspergillus niger- Incubation was done for 5days at 25^0c

Results:-
Krimighna Kwatha(Decoction prepared from *Krimighna Mahakashaya Gana*[6]) and *Taila*(oil prepared from *Krimighna Mahakashaya Gana*[6]) did not show any zone of inhibition at 10, 25, 50, 100,150 µl concentrations on *Aspergillus niger, Candida albicans, Microsporum canis,Trychophyton tonsurans*. It is observed that activity of Standard drug Amphotericin B and Fluconazole is significant against microbes. And the standard drug Fluconazole showed zone of inhibition of 12mm at 100 mg/ml and 10 mm at 100 mg/ml on *Aspergillus* and *Candida albicans* respectively. Standard drug Amphotericin B showed zone of inhibition of 09 mm at 100 mg/ml and 10 mm at 100 mg/ml on *Microsporum canis,* and *Trychophyton tonsurans* respectively.

Discussions:-
Eventhough antimicrobial activity for the individual drugs of *Krimighna Maha KashayaGana* has been proved earlier, also the antibacterial activity and activity against intestinal worms for the formulation has been already proved.In present study the the formulation as a whole was ineffective against the fungal strains used. Reason may be Microorganism procured may be genetically resistant or virulent. The dosage and concentrations of the preparations adopted for the study may not be sufficient to overcome the virulence of organisms. The microbial load taken for the study might be too high for the dosage form .Dosage form adopted may not be effective for the present fungal strains. Dosage form adopted would be effective in bacterial strains or against intestinal parasites not against fungal strains.The study helped to generate pharmaceutical and analytical standards for *Krimighna Mahakashaya Gana Churna, Kalka* and *Taila*

Conclusion:-
The present dosage forms could be tried for other fungal strains for fetching positive results.This research would be an eye opener to restrict the *Krimighna Mahakashaya Gana* against dermatophytes.

Declarations
Ethical Approval and Consent to participate"Not applicable"
Consent for publication
Consent from main and corresponding authors are available .
Availability of supporting data"Not applicable"
Competing interests"Not applicable"

Funding"Not applicable"
Authors contribution This is a Research work carried by Main author with the guidance from corresponding authors and send for publication with their approval.
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Table 1:-Organoleptic features of *Kwatha*(decoction) and *Taila*(oil) Prepared from *Krimighna Mahakashaya Gana*(Combination of 10 drugs)

Parameters	**Kwatha(decoction)**	***Taila*(oil)**
Colour	Brown colour	Greenish blue
Odour	Characteristic	Pleasant
Taste	Acrid	Acrid
Consistency	Viscous	Highly Viscous

Table 2:-Physico-chemical parameters of *Krimighna Mahakashaya Gana Kwatha Churna*(Powder for decoction combination of 10 drugs)

Parameters	**Results n=3 %w/w**
Loss on drying	5.995
Total ash	10.075
Acid insoluble ash	0.997
Water soluble ash	2.495
Alcohol soluble extractive	2.994
Water soluble extractive	8.053

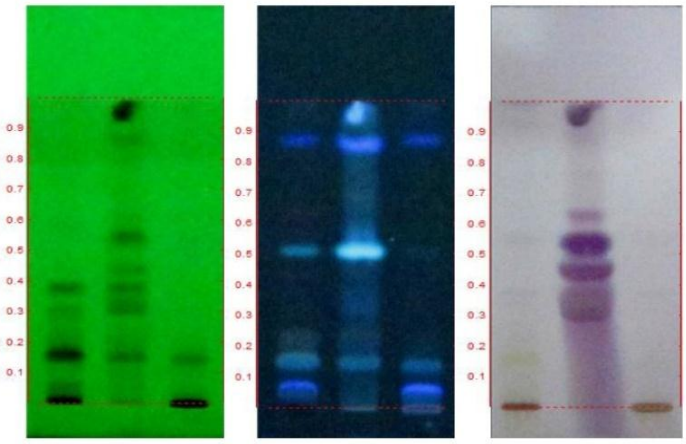
Table 3:-Physico-chemical parameters of *Krimighna Mahakashaya Gana Kwatha* (Decoction prepared from combination of 10 drugs)

Parameters Results	**n=3 %w/w**
Refractive index	1.33669
Specific gravity	1.0105
Viscosity	2.7939

Table 4:-Physico-chemical parameters of *Krimighna Mahakashaya Gana Taila*(oil prepared from combination of 10 drugs)

Parameters Results	**n=3 %w/w**
Refractive index	1.4697
Specific gravity	0.9177
Viscosity	83.9885
Rancidity	Fat is not oxidized
Acid value	1.9615
Saponification value	154.3718
Unsaponifiable matter	2.1526
Iodine value	92.3921
Peroxide value	7.5908
Boiling point	258ºC

HPTLC Documentation of the Preparations



Track 1- Alcohol extract Krimigha Kashaya Churna prepared from drugs of Krimighna Maha Kashaya gana(Combination of 10 drugs)

(sample-1) - 8 µl

Track 2– Chloroform extract of ***Krimighna Taila*(oil)prepared from drugs of *Krimighna Maha Kashaya gana*(Combination of 10 drugs)**

(sample-2)- 8 µl

Track 3– Chloroform extract of ***Krimighna Kashaya*(decoction*)* prepared from drugs of *Krimighna Maha Kashaya gana*(Combination of 10 drugs)**

(sample-3)- 8 µl

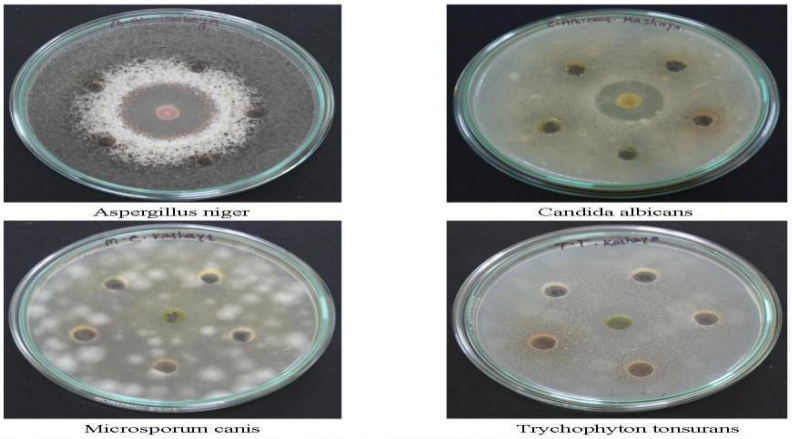
Solvent system:

Toluene: Ethyl acetate (7:1)

Experimental study of the preparations on Dermatophytes.

I. Agar disc diffusion method adopted for *Krimighna Kwatha* (decoction) on Dermatophytes against Standard drug.

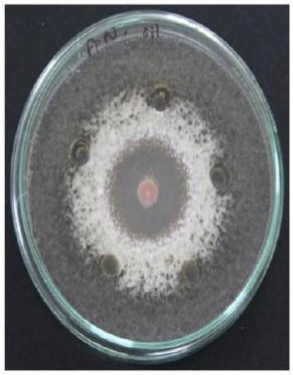
In vitro study of Kwatha



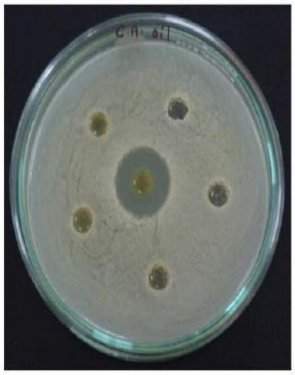
In vitro study of Ethanolic extract of Krimighna Mahakashaya Churna

II. Agar disc diffusion method adopted for *Krimighna Taila* (oil) on Dermatophytes against Standard drug

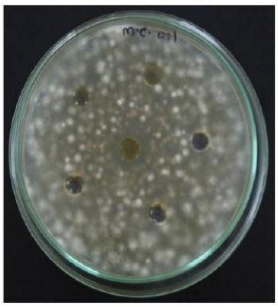
In vitro study of Taila



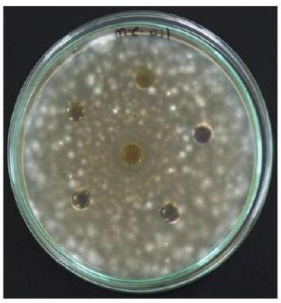
Aspergillus niger



Candida albicans



Microsporum canis



Trychophyton tonsurans

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