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PHARMACEUTICO-ANALYTICAL EVALUATION AND IN-VITRO ANTIFUNGAL ACTIVITY OF KHADIRA KWATHA AND KHADIRA GHANAVATI AGAINST CANDIDA ALBICANS

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ABSTRACT

Background: Khadira (Acacia catechu Willd.) is an important Ayurvedic drug traditionally indicated in the management of Kushtha. Khadira Kwatha is a classical liquid formulation, while Khadira Ghanavati is a concentrated solid dosage form prepared from the decoction. **Aim**: To evaluate and compare the pharmaceutical and physicochemical characteristics of Khadira Kwatha and Khadira Ghanavati and assess their in-vitro antifungal activity against Candida albicans. **Materials and Methods:** Khadira Kwatha was prepared using 100 g of coarse Khadira heartwood powder and 1600 mL of water, reducing the volume to approximately one-eighth of the initial quantity. The decoction was further concentrated to obtain Ghana, which was processed into Ghanavati. Both formulations were evaluated for organoleptic characteristics, physicochemical parameters, and thin-layer chromatography (TLC) and In-vitro antifungal activity against Candida albicans, **Results:** Both formulations showed acceptable physicochemical and microbiological quality. Khadira ghanavati exhibited more antifungal activity than Khadira Kwatha **Conclusion:** Khadira Ghanavati showed greater inhibition than Khadira Kwatha. Also it is easy to administer and store. Further experimental and clinical studies are required to confirm its effectiveness in the management of kushtha.

KEYWORDS: Khadira, Acacia catechu, Kwatha, Ghanavati, Candida albicans, antifungal activity, pharmaceutical evaluation, TLC.

INTRODUCTION

In Ayurveda, *Kushtha* is a group of skin disorders associated with the vitiation of Tridosha (Vata, Pitta, and Kapha) and the involvement of Twak, Rakta, Mamsa, and Lasika, Acharya Charakahas described Kushtha is a Tridoshaja Vyadhi.[1] Charaka Samhita advocates repeated Shodhana therapies such as Vamana and Virechana followed by Shamana Chikitsa using Kushthaghna drugs like Khadira, Bakuchi, and Nimba emphasized for the successful management of Kushtha.[2] Candida species, one of the most common fungal pathogens, can cause everything from simple mucocutaneous infections to severe systemic diseases. Because fungal infections and skin disorders are becoming more prevalent in the society, Kushtha was selected for this investigation.[3] Khadira is considered one of the most important and specific drugs. Ayurveda classics have given prime importance to Khadira in the management of Kushtha. Acharya Charaka has mentioned Khadira in Kushthaghna Mahakashaya, indicating its special action against skin diseases.[4] Khadira's antifungal and anti-inflammatory qualities may lessen erythema, irritation, fungal colonization, and recurrent infection in mucocutaneous diseases.[5]

Kwatha Kalpana has certain limitations such as difficult preparation, shorter shelf life, unpleasant taste, larger dose requirement, and poor patient compliance.

To overcome these drawbacks, Khadira Ghanavati has been developed as a modified dosage form. Ghanavati possesses several advantages over Kwatha Kalpana such as easy administration, better palatability, precise dosage, portability, longer shelf life, and improved patient acceptability and compliance. Due to the concentrated form of the drug, it may also provide enhanced therapeutic efficacy.

There is always a need to evaluate a new drug dosage form for various criteria like potency, applicability, stability, quickly absorbable, pleasant and safe. The growth of research and sophisticated technology has made it possible to create a standard database of herbs, minerals, metals, and even finished goods. This would not only give Ayurvedic drugs a scientific foundation and respectability, but it will also aid in the globalization of Ayurveda.

NEED FOR TRANSFORMATION OF KWATHA INTO GHANAVATI

In today’s time, patients prefer treatments that are simple, clean, and easy to use. There is also a need for formulations that remain stable for a longer time and maintain consistent quality. Kwatha, although effective, does not always meet these requirements, because of its short

shelf life, inconvenience in use, its wider use becomes limited. To overcome these issues, there is a need to convert kwatha into more suitable forms that is Ghanavati, such modifications can help in improving stability, convenience, and overall acceptability of the treatment.

Antimicrobial activity of Khadira[6]

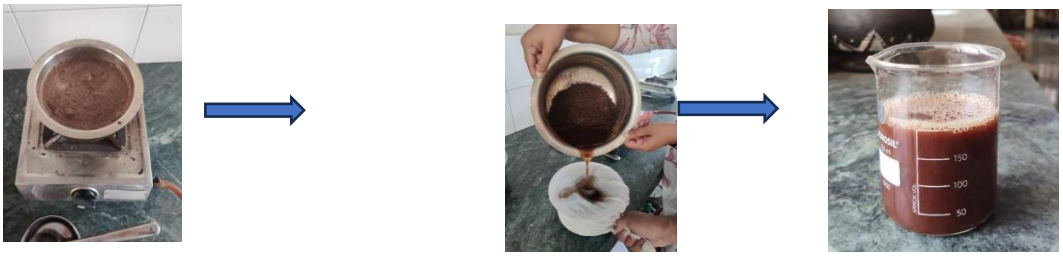
Acacia catechu Willd leaves Extract is found to possess broad-spectrum antimicrobial activity Results showed AC has antimicrobial activity by inhibiting common human pathogenic organisms like Staphylococcus aureus (Gram positive), Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Salmonellatyphi (Gram negative) and fungi like Candida albicans, Aspergillus niger supporting its use in traditional medicine. Acacia catechu Willd leaves, Bark, root extract also possesses Anti mycotic activity. The bark extract showed an inhibitory effect on the growth of fungi such as Piricularia oryzae and Colletotrichum falcatum.

Wound Healing and Tissue Protectant Properties *Khadira* actively facilitate wound healing, a property attributed to its astringent, antibacterial, antifungal, and *Ropan* (healing) characteristics. It promotes the recovery of wounds by stimulating fibroblast collagen production and reinforcing collagen fibers. Its antimicrobial properties prevent potential infections, supporting the healing process, while its astringent effect causes contraction of skin cells, diminishing inflammation.[7] Epicatechin, a major constituent, has been confirmed to play a significant role in diabetic wound healing by stabilizing human inducible nitric oxide synthase (iNOS)[8]

MATERIAL AND METHOD

1. Preparation of Khadira Kwatha[9]

Ingredients	Quantity
Khadira	**100** gms
Water	1600ml



The raw drug was collected from local market and identified. The raw drugs were pounded into coarse powder. 100gms of Khadira bharad was taken in a wide mouthed stainless-steel vessel. It was added with 1600 ml of water and heated in a gas stove. Constant mild heat was applied to facilitate the evaporation and intermediate stirring was carried out till reduced to 1/8th of the initial quantity. After desirable reduction in volume (1/8th – 200ml), the Kashaya was filtered through four folded cotton cloth and collected in a separate vessel for further processing. The residue remained above cloth were discarded. It was observed that initially the liquid was light brown in colour and bitter in taste. The prepared Kashaya had the odour of the drugs added. Evaporation was started at 70 º C, which was aggravated on stirring. The Kashaya on boiling was light brown colour in the initial stage, which was gradually turned to dark brown in colour. The residue after preparation was odourless and tasteless.

2. Preparation of Khadira Ghanavati[10]

Ingredients	Quantity
Khadira	100gms
Water	1600ml



Khadira Ghanavati was prepared by taking 100 g of coarse powder of Khadira (Acacia catechu Willd.) and adding 1600 mL of water. The mixture was boiled and reduced to one-eighth of its initial volume, approximately 200 mL, to obtain Khadira Kwatha. The decoction was then filtered to remove the solid, The Kashaya was heated on Gas stove and temperature was maintained in between 70°C-75°C. Continuous stirring of Kashaya was done to avoid burning of Kashaya. After that it was converted into semisolid stage and heating process was stopped This concentrated mass was allowed to cool, rolled into small pills of uniform size, and dried appropriately to obtain Khadira Ghanavati. The prepared Ghanavati was stored in a clean, dry, airtight container.

It was observed that after some time of boiling mild sticky nature was observed when rubbed between two fingers. After some time of heating stickiness of the liquid and adhesiveness to

the vessels was increased. Thickness of liquid and adhesiveness to vessel was gradually increased. dark brown coloured, semi-solid material was converted into blackish brown coloured solid material.

The following parameters were employed for this study, which is mentioned in Ayurvedic Pharmacopoeia of India.

1. Organoleptic parameters
2. Physicochemical analysis

The following parameters were compared of Khadira Kashaya and Khadira Ghanavati

a) pH value[11]
b) Total Solids
c) Loss on drying[12]
d) Alcohol soluble extractive

Viscosity and Specific gravity of the decoction was checked, while the disintegration time, hardness, friability of the tablet, total ash and acid insoluble ash was checked to determine the stability of the dosage for. Also, TLC.[13] and antifungal test was done by using agar well diffusion method.[14]

OBSERVATIONS AND RESULTS

- **Pharmaceutical study**
  - **Organo Leptic Characters of *Khadira Kwatha***

Sr.No	Characters	observation
1	Physical appearance	Liquid
2	Touch	Cool, watery
3	Colour	Brownish black
4	Taste	Tikta, kashay
5	Odour	Characteristic smell of *Khadira*

  - **Organo Leptic Characters of *Khadira Ghanavati***

Sr.No	Characters	Observations
1	Physical appearance	Solid
2	Touch	Hard
3	Colour	Black
4	Taste	Tikta,kashay
5	Odour	Characteristic smell of *Khadira*

Sr.no	Parameter (observations)	*Khadira Kwatha*	*Khadira Ghanavati*
1	Weight of Raw Drug (Khadira Bharad)	100 g	100 g
2	Amount of Water Added	1600 ml	1600 ml
3	Final Yield Obtained	200 ml kwatha	16 gm ghana
4	Total Loss	1400 mL (water evaporated	84 gm
5	% Loss	87.5%	84%
6	Time Taken	1 H 20 M	2 H 50 M

- **Analytical Study**
  - **Physiochemical properties of *Khadira Kwatha***

Sr.No	Parameters		values
1	pH		5.3
2	Ash value		7.32%
3	Water extractive	soluble	23.05%
4	Alcohol extractive	solube	1.39%
5	Acid insoluble ash		1.56%

  - **Physiochemical properties of Khadira Ghanavati.**

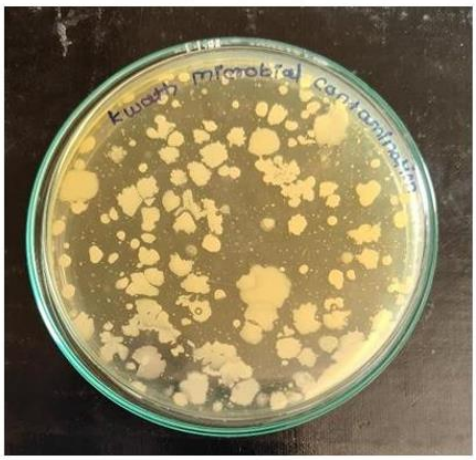
Sr.No	Parameters	values
1	Ph	4.8
2	Hardness	5.2Kg/cm2
3	Friability test	0.45%
4	Disintergration test	18 min
5	LOD	3.4%
6	Ash value	5.8%
7	Alcohol soluble extractive	15.2%
8	Water soluble extractive	28.4%
9	Acid insoluble ash	0.8%

Organoleptic evaluation revealed characteristic color, odor, appearance, and consistency in both samples. Khadira ghanavti showed comparatively improved aroma and darker appearance.

Physicochemical evaluation demonstrated acceptable analytical parameters and confirmed proper pharmaceutical processing.

- **Microbial contamination**

Aerobic Microbial Count



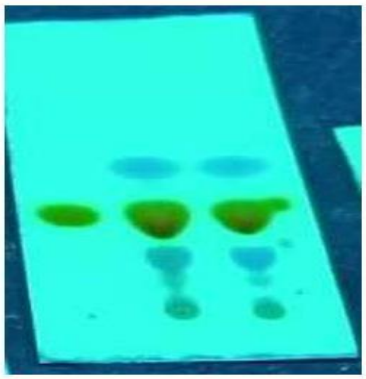


Sr.no	Sample	No of Colonies	CFU/ml	Permissible Limit (API/WHO)	Result
1	Sample- *Kwatha*	1120	11200	Not more than 10^5 CFU/g or mL	Within Limit
2	Sample *ghanavati*	1020	1020	Not more than 10^5 CFU/g or mL	Within Limit

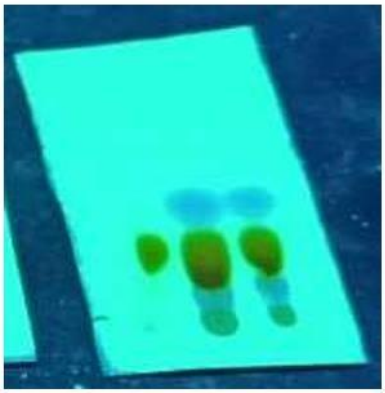
Total microbial count analysis revealed absence of microbial contamination in both samples.

Observation of TLC

Sr. no	Name of plant	Mobile Phase	Ratio
1	Sample - *Khadira Ghanavati*	Toluene:Ethyl acetate:Formic acid	6:3:1
2	Sample- *Khadira Kwatha*	Toluene:Ethyl acetate:Formic acid	6:3:1



Khadira Ghanavati



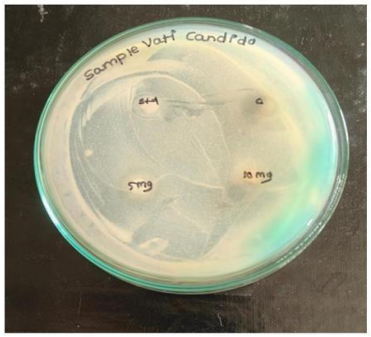
Khadira Kwatha

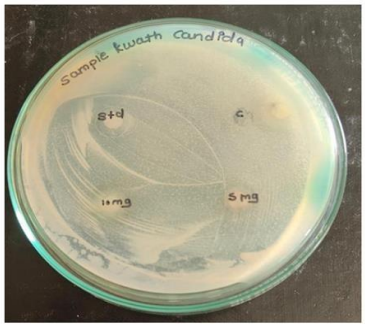
RF Value = distance traveled by the compound / distance traveled by the solvent front

1. **Samples - Gallic Acid RF Value =3.2\ 4.6 = 0.69**
2. **Sample - *Khadira Ghanwati* RF Value = - 3.5\ 4.8 = 0.72**
3. **Sample – Khadira Kwatha RF Value = - 2.9\ 4.5 = 0.6**

TLC analysis confirmed the presence of important phytochemical constituents in both formulations.

ANTIFUNGAL ACTIVITY AGAINST CANDIDA ALBICAN





Sr.no	Sample	Concentration	Zone of Inhibition (mm) Candida albicans
1	Standard Fluconazole	1 mg/ml	10
2	Sample -Vati	5 mg/mL	02
		10mg/mL	03
3	Sample -Kwatha	5 mg/mL	01
		10mg/mL	02

Antifungal study demonstrated that both samples possessed inhibitory activity against candida albicans, with *Khadira ghanavati* showing slightly higher zone of inhibition than *Khadira kwatha* under the given test conditions.

DISCUSSION

The present study was conducted to evaluate the pharmaceutical, analytical, and in-vitro antifungal activity of Khadira (*Acacia catechu* Willd.) Kwatha and Ghanavati against *Candida albicans*. Khadira is traditionally used in Ayurveda for the management of Kushtha, providing the rationale for investigating its antifungal potential.

Khadira Kwatha was prepared using 100 g of coarse Khadira powder and 1600 mL of water, which was reduced to approximately one-eighth of its initial volume. The decoction was

further concentrated to prepare Khadira Ghanavati. The two formulations were subsequently evaluated for their physicochemical characteristics and chromatographic profiles.

The analytical results showed that the pH of Kwatha and Ghanavati was 5.3 and 4.8, respectively. The total ash values were 7.32% for Kwatha and 5.8% for Ghanavati, while the water-soluble extractive values were 23.05% and 28.4%, respectively. These variations indicate differences in the measured analytical parameters of the two formulations. TLC analysis also demonstrated differences in their reported chromatographic profiles, although further confirmatory analysis is necessary for the identification of individual constituents.

In the in-vitro antifungal study, Khadira Kwatha produced inhibition zones of 1 mm and 2 mm at concentrations of 5 mg/mL and 10 mg/mL, respectively. Khadira Ghanavati produced inhibition zones of 2 mm and 3 mm at the corresponding concentrations, whereas Fluconazole, the standard drug, produced an inhibition zone of 10 mm. Thus, Ghanavati showed numerically greater inhibition than Kwatha under the tested conditions. However, the inhibition zones of both formulations were small compared with the standard drug, and the findings should be interpreted cautiously.

Overall, the study provides preliminary comparative data on the pharmaceutical and analytical characteristics of Khadira Kwatha and Ghanavati, along with their observed in-vitro activity against *Candida albicans*. Further investigations using standardized methods, adequate replicates, and statistical analysis are required to confirm the findings and assess their reproducibility.

CONCLUSION

The present study evaluated Khadira (*Acacia catechu* Willd.) Kwatha and Ghanavati through pharmaceutical, analytical, and in-vitro antifungal investigations. Differences were observed in their physicochemical parameters and TLC profiles. Both formulations produced small inhibition zones against *Candida albicans*, with Ghanavati showing numerically greater inhibition than Kwatha at the tested concentrations, although the activity was lower than that of Fluconazole, the standard drug.

From a pharmaceutical perspective, Khadira Ghanavati offers practical advantages over Kwatha, including ease of administration, convenience in handling and transportation, and the potential for improved storage stability due to its concentrated solid dosage form. Its

potentially longer shelf life is an additional advantage, although this requires confirmation through appropriate stability studies.

Hence, **Khadira Ghanavati may be considered a more convenient and practical dosage form than Khadira Kwatha**, with numerically greater in-vitro inhibition under the tested conditions. However, the observed antifungal activity remains preliminary. Further studies involving standardized preparation, stability testing, adequate experimental replication, and statistical analysis are necessary to validate these findings and determine the potential of both formulations for future research.

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