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Phytochemical analysis of useful parts of *Barbari* (*Ocimum basilicum* Linn.) and its evaluation for Anti-Microbial Activity

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ABSTRACT

In recent decades, the interest in evaluating therapeutic effects of plants has increased d. Phyto-medicines have been shown to be good alternatives to synthetic chemical antimicrobial agents because of the serious side effects, antimicrobial resistance and the emergence of previously uncommon infections due to inappropriate or widespread overuse of antimicrobials. *Barbari* is a commonly available drug and have also been used as a folk remedy to treat various ailments. Hence Phytochemical Analysis of useful parts of *Barbari* (*Ocimum basilicum* Linn.) and its evaluation for anti-microbial activity were taken for study. The objectives of the study are Pharmacognostic study of useful parts in *Barbari*, Phytochemical analysis of useful parts in *Barbari*, Qualitative analysis of Phyto-elements present in the drug and Anti-microbial activity of *Barbari* on certain strains of microbes. Extraction of *Arka* from both leaves & seeds were done as per the norms mentioned in classics. Phytochemical Analysis of different extracts is done. Determinations of volatile oil percentage and chemical finger printing by GC-MS and HPLC of both the *Arka* have been conducted, along with their antimicrobial activities evaluated. The Results obtained are negative for the presence of Euginol by GC-MS & HPLC. Both the samples were found to be inactive against tested bacterial and fungal strains.

Key words: *Barbari, Ocimum basilicum, Anti-Microbial Activity.*

INTRODUCTION

The genus Ocimum is represented by over 50 species of herbs and shrubs. The plant is rich in volatile oils, the antimicrobial activity of which is well known. The Lamiaceae family members are the most employed medicinal plants as a worldwide source of spices and also as a consolidated source of extracts with strong antimicrobial and antioxidant properties.[1] Basil oil has also found a wide application in perfumery as well as in dental and oral products. They have also been used as a folk remedy to treat various ailments such as; poor digestion, nausea, gastro-enteritis, migraine, insomnia, gonorrhea. Externally, they have been applied for the treatment of acne, loss of smell, insect stings, snake bites and skin infections.[2]

The antimicrobial properties of many plants have been investigated by a number of researchers worldwide. Around 12,000 aromatic substances have been isolated asphenolic, Terpenoids, nitrogen compounds, vitamins and some other endogenous metabolites. These substances serve as plant's defense mechanism against microbes, insects & herbivores. Multiple drug resistance in both human and plant pathogenic micro-organisms have been

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developed due to the indiscriminate use of antimicrobial drugs commonly used in the treatment of infectious diseases. *Barbari* is a commonly available drug and used by Ayurvedic physicians, but has not been studied elaborately. The present study has been undertaken to establish the antimicrobial property of *Ocimum basilicum*. Our ancient scholars were aware of the existence of microorganisms causing diseases since Vedic period. There are many references pertaining to *Jivanuvada* (bacteriology) in ancient literature such as Rigveda and Atharvaveda. In Atharvaveda, various words are used for *Krimi* which have their own specific meanings.

Nearly hundred types of organisms have been described there, some are said to be *Drsta* (Macroscopic) and others are *Adrsta* (Microscopic).

Objectives of the Study

- Phytochemical analysis of useful parts of *Barbari*.
- Qualitative analysis of Phytoelements present in the drug.
- Anti-microbial activity of *Barbari* on certain strains of microbes.

Observations and Results

Determination of Ash Value

Table 7: Ash Values of Seeds and Leaves

SN	Ash Value	Seeds	Leaves
1.	Total ash	3%	5%
2.	Acid insoluble ash	*1%*	0.5%
3.	Water soluble ash	5%	3%

Extractive Values

Table 8: Extractive Values of Seeds and Leaves

SN	Solute Used	Seeds	Leaves
1.	Water	3%	11%
2.	Alcohol	8%	5.9%
3.	Methanol	5%	6.6%

Qualitative Estimation of Essential Oil from *Ocimum Basilicum* (*Arka*)

- Leaves - 1.5% (v/w)
- Seeds - 0.8% (v/w)

Extraction of Arka from Leaves & Seeds through Distillation

Phytochemical Analysis of Seeds

Table 9: Phytochemical analysis of different extracts and *Arka* of Seeds

Tests	Methanol Ext.	Alcohol Ext.	Aqueous Ext.	Arka	Observations
Saponins	-ve	-ve	-ve	-ve	No froth formed
Tannins	-ve	-ve	-ve	-ve	No blue colour formed
Flavonoids	+ve	+ve	+ve	-ve	Yellow colour formed
Steroids	-ve	-ve	-ve	-ve	No green florescence

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Terpenoids	+ve	+ve	+ve	+ve	Interfere layer shows brick red colour
Cardiac glycosides	+ve	-ve	-ve	-ve	No blue colour or blue ppt.
Alkaloids	+ve	+ve	-ve	-ve	Appearance of orange red colour
Reducing sugars	+ve	-ve	+ve	-ve	Black precipitate formed

Phytochemical Analysis of Leaves

Table 10: Phytochemical analysis of different Extracts and Arka of leaves.

Tests	Methanol Ext.	Alcohol Ext.	Aqueous Ext.	Arka	Observations
Saponins	-ve	-ve	+ve	+ve	Froth formation
Tannins	-ve	-ve	-ve	+ve	Blue colour formed
Flavonoids	+ve	+ve	+ve	-ve	Yellow colour formed
Steroids	-ve	-ve	-ve	-ve	No green florescence
Terpenoids	+ve	+ve	+ve	+ve	Interfere layer shows brick red colour
Cardiac glycosides	-ve	-ve	-ve	-ve	Brick red to orange colour
Alkaloids	-ve	-ve	+ve	+ve	Appearance of orange red colour
Reducing sugars	-ve	+ve	+ve	+ve	Black ppt formed

Results

Chemical finger printing by GC-MS & HPLC

Euginol is not present in the given Ayurvedic sample.

Antimicrobial Study

Antimicrobial activity of M2 (seeds)

Table 11: Anti-microbial activity of Seeds

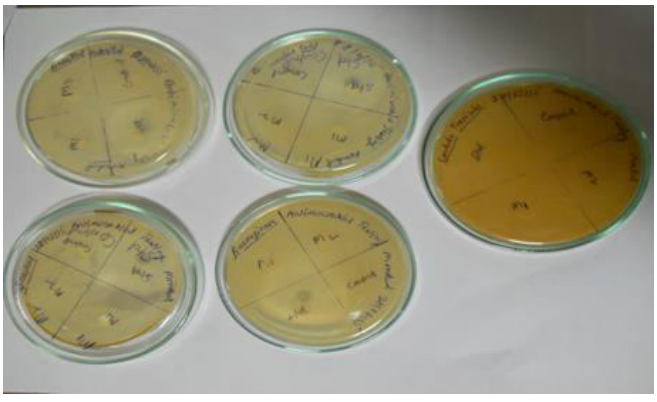
Name of tested organism	Zone Diameter (mm) of Growth Inhibition		
	Test Drug	Control	Standard Drug
E. Coli	-	-	**27**
P. aeruginosa	-	-	**36**
B. subtilis			**32**
S. aureus	-	-	**38**
C. tropicalis	-	-	**8**

Antimicrobial activity of M1 (leaves)

Table 12: Anti-microbial activity of leaves

Name of tested organism	Zone Diameter (mm) of Growth Inhibition		
	Test Drug	Control	Standard Drug
E. Coli	-	-	**27**
P. aeruginosa	-	-	36
B. subtilis			**32**
S. aureus	-	-	**38**
C. tropicalis	-	-	**8**

Petri Plates – Anti-Microbial & Anti- fungal Activity



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Results

Given samples were found to be inactive against tested bacterial and fungal strains.

DISCUSSION

In this study *Barbari* seeds and leaves have been selected as the useful part of the plant is *Patra* and *Beeja* as mentioned in classics.[3] Also the volatile oil is found more when compared to other parts of the plant. Analytical study of *Barbari* is needed to know its chemical composition which in turn help in understanding the activity of the drug, as the principles of *Dravya, Guna, Veerya, Vipaka, Prabhava* cannot be measured easily. Knowledge of Phyto-constituents helps in evaluating the action of drug. With this view following studies were carried out on *Barbari* - Distillation, Phytochemical analysis, HPLC, GCMS.

Total Ash value

It was 3% in seeds and 5% in leaves. Ash value is the residue remaining after incineration of the crude drug. This represents inorganic salts present in the drug. Carbonates, Bicarbonates, Nitrates, Chlorides etc. The ash value is within the normal range as provided in Quality standards of Indian medicinal plants.

Acid insoluble ash

Acid insoluble ash of the given sample *Barbari* is 0.5%. This indicates ash insoluble in dilute HCl which signifies adhering sand and silica present. If acid insoluble ash is more than 6% then we can consider that there is excess of sand and silica, these may accumulate in various systems as they are not digested in acid media and gets deposited in various organs. *Barbari* leaves and seeds were collected and dried under shade in a room. Hence there is no contact with sand and silica. Therefore, percentage was within the normal range.

Alcohol soluble extraction

It was 20% in seeds and 5.9% in leaves indicates the presence of alcohol soluble organic compounds like alkaloids, carbohydrates, glycosides.

Water soluble extracts

It was 3% in seeds and 11% in leaves. This applies for both organic and inorganic compounds like water soluble alkaloids, tannins, sugar, gums, mucilage, glycosides.

Chemical analysis

Saponins, tannins, Terpenoids, alkaloids and reducing sugar were present in leaf extract (*Arka*), but only Terpenoids are present in seed extract (*Arka*).

Phytochemical study

Preliminary phytochemical analyses of the sample revealed the positive results for Saponins, tannins, alkaloids, Terpenoids, tannins and negative results for others in leaf *Arka*. Chemical constituents present in *Arka* (leaves and seeds) like, Alkaloids, Tannins have shown antimicrobial and antifungal activities as mentioned below;

- Saponins may serve as anti-feedants and to protect the plant against microbes and fungi. They are often bitter to taste, and so can reduce plant palatability.
- Tannins act as a barrier for micro-organisms like bacteria and fungi to protect the plants.
- Alkaloids have a wide range of pharmacological activities including antimalarial, antiasthma, anticancer, vasodilator, anti arrhythmic, analgesic, and antibacterial, antihyperglycemic activities.[4]

Antimicrobial study

- For the antimicrobial and antifungal, study the extract was supposed to be concentrated before the experiment. Which is impossible as my study was on *Arka*; the 60% water extract. Only 0.8% and 1.5% of volatile oil was found respectively in seeds and leaves.
- The dose of *Arka* is 12 to 24 ml (as per the classics), but in the present study only 20µl was taken which is the maximum limit to load in Petri dish plates.

Probable mode of action

While describing the causes for the *Utpatti* of *Krimi, Acharyas* have mentioned the *Ahara* which are having

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the properties like *Madhura Rasa; Guru, Snigdha* and *Manda Gunas*; *Sheeta Veerya* and *Madhura* or amla *Vipaka*. *Ksheera*, *Guda*, *Tila*, *Matsya*, *Pishtanna*, are few examples for the causes of *Krimi Utpatti*.[7]

Barbari possesses opposite qualities to that of *Krimi Nidana* i.e. *Prakruti Vighata Chikitsa*.[5] As *Katu* and *Tikta Rasas* are having opposite actions to that of *Madhura Rasa*, *Tikta* and *Katu Rasa* has best *Krimighna* property.[6] *Ruksha*, *Teekshna* and *Vidahi* properties are quite opposite to that of *Guru*, *Snigdha* & *Manda Gunas*. *Barbari* has *Ushna Veerya* which destroys the *Sheeta* which is well suited for the growth of *Krimi*.

Rasa, Guna, Veerya, Vipaka, and *Doshaghnata* of *Barbari* are opposite to that of causative factors for the *Krimi Utpadana*. Here *Barbari* acts as Krimighna and may also destroy the microbial activity.

Conclusion

Antimicrobial activity may be failed due to various factors like incorrect drying, exposure to heat, evaporation of volatile oil during or after distillation, due to incorrect collection and storage of *Arka*. As volatile oils like Euginol are the most important antimicrobial agents their absence might have influenced the 'no growth zone' in the antimicrobial study. Incorrect antimicrobial methods and concentration variation or microbes might be resistant to the drug *Barbari*. Most drugs that are ineffective in invitro prove to be effective in vivo. Hence further study is needed on this drug *Barbari* to prove its efficacy on the microbes and the diseases produced by the microbes. Scope for further study are, it should be carried out in different culture Medias, In vivo studies can be carried out for further assessment, The pharmaceutical standardization of the formulation can be concentrated, Studies can be conducted for the whole plant to obtain better results, Chemical fingerprinting of other active constituents can be carried out, Drug should be collected from different habitat and in different seasons for successful evaluation of antimicrobial activity.

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