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Review on Pharmacognostical, Phytochemical and Pharmacological Evaluation of Osbeckia Muralis Naudin

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ABSTRACT: The plant 'Nela Nekkarika' in Kannada, 'Cherkulathi' in Malayalam and 'Cen-Thumbai' in Tamil is botanically identified as Osbeckia muralis Naudin belongs to the family Melastomaceae. This is an herb endemic to Western Ghats.In this review article focus to explore the pharmacognostical ,phytochemical and pharmacological importance of Osbeckia muralis. pharmacognostical study of is undertaken. For the purpose of standardization, macroscopic, microscopic and powder microscopic studies has been carried out. The morphological study shows that the herb is a 30 cm tall less branched hirsute herb with elliptic shaped leaves tetramerous flowers and purple petals. The microscopic study of the stem and the leaf has been carried out which shows chlorenchyma cells, glandular trichomes, multicellular covering trichomes, calcium oxalate crystals, pallisade parenchyma and spongy parenchyma etc. The powder microscopic study shows multiserriate trichome, glandular trichome, pith parenchyma etc. This study can help for the standardization and authentication of the genuine drug. The scientific exploration for the proper identification has been carried out by using Physico chemical and Phytochemical analysis including HPTLC. The study helped to get the moisture content, Ash values, extractive values in different solvents, and HPTLC
etc. The different peaks of Rf value help to identify various components. The study reports the presence of Protein, Carbohydrates, Tannins,Flavanoids, Alkaloids, Chloride, Sulphate, Potassium, Sodium.Scientific exploration of the drug has been done by acute oral toxicity study and Anti-bacterial Study. Acute oral toxicity study showed no signs of toxicity or behavioral changes. Antibacterial study with Disk diffusion method showed the action of Osbeckia muralis Kashaya in Klebseilla pneumoniae was positive.

Keywords – Osbeckia muralis Naudin,Pharmacognostical study, Phytochemical study, Physicochemical study, HPTLC Toxicity study, Anti-bacterial Study

I. INTRODUCTION

Plants are the important source of medicine, have a key role in world health.Medicinal plants are the important source of therapeutics known as active constituents used as curative aids, also they have a great involvement in the maintenance of health system in all over the world. [1]

The vast majority of people on this planet still rely on their traditioinal material medica (medicinal plants and other materials) for their everyday health care needs.It is also a fact that one quarter of all medicinal prescription are formulation based on substances derived from plants there for the active ingredients from the herbs have high healing effect. [2]

Pharmacognostical and phytochemical studies are helpful in identification and standardization of plant material. Pharmacological study aims to evaluate its toxicity and its curative property, This information about toxicity study is useful to determine the relevance of the test for the protection of the human health and the environment, and will help in the selection of an appropriate starting dose.[1]

Osbeckia is a genus of plants in the family Melastomataceae. It was named by Carl Linnaeus for the Swedish explorer and naturalist Pehr Osbeck (1723–1805).

Osbeckia muralis naudin,is an endemic herb of Western Ghats.There is no reference about the drug in any of the classical Ayurvedic books. Some folklore practitioners from the foot hills of Western Ghats especially in Dakshina Kannada District, are using the leaves of Osbeckia muralis Naudin. which is commonly known as 'Nela

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Nakkarika'' in Kannada, indifferent forms like .

Swarasa and Kashaya mainly in cough.[3]

Fig:1: Osbeckia muralis Naudin

VERNACULAR NAMES: .[3,4,5]
In India
Common Name: Wall Osbeckia
Kannada: Jogi gida,Gidda nekkare,Gidda nekkarika
Malayalam:Centumpa,Cherukulathi,Kunjathirai
Marathi:Gulbeki
Sanskrit: Tinisah
Tamil:Tumpai
Telugu:Burada alli
Tulu:Dai nekkare

GEOGRAPHICAL SOURCE; .[3,4,5]
Maharashtra: Kolhapur, Nasik, Pune, Raigad, Ratnagiri,Thane
Kerala: Alapuzha,Kannur,Kasaragod, Kollam, Kottayam,Malapuram,Palakkad, Pathanamthitta, Thrissur
Tamil Nadu: Coimbatore, Nilgiri, Tirunelveli, Theni

SCIENTIFIC REPRESENTATION:.[3,4,5]
Kingdom : plantae
Clade : Tracheophytes
Clade : Angiosperms
Clade : Eudicots
Clade : Rosids
Order : Myrtales
Family : Melastomataceae
Genus : Osbeckia L.

II. AIM AND OBJECTIVE:

In this review collect the pharmacognostical, phytochemical and pharmacological data of Osbeckia Muralis

1. To explore the identity of Osbeckia muralis by Macroscopic study
2. To evaluate Osbeckia muralis by Microscopic study
3. To evaluate the identity of Osbcekia muralis by powder microscopy
4.To evaluate the active constituents of Osbcekia muralis by phytochemical screening
5. To evaluate the toxicity and ailment property of Osbcekia muralis by pharmacological study

III. METHODOLOGY [6,7,8]

1. PHARMACONOSTIC STUDY

To explore its identity, pharmacognostical study of Osbeckia muralis Naudin is undertaken. For the purpose of standardization, macroscopic, microscopic and powder microscopic studies has been carried out.

a) Botanical/Macroscopic Description

The macroscopic study is the morphological description of the plant parts which was crried out by a naked eye placing the plant material on a white paper surface.Organoleptic features such as shape, size, colour, odour,taste of leaves,flowers and fruits were evaluated.

b) Microscopy:

Material (Leaf & Stem) was preserved in fixative solution as FAA (ie; Formalin:Acetic acid:70% Ethyl alcohol, 5:5:90). Take a thin

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transverse section from the specimen and stained with saffranine then mount with a drop of glycerin-water and focus through microscope

c) Powder Microscopy:

Shade dried leaves were finely powdered and take a pinch of powdered sample in a microscopic slide with a suitable staining agent (Phluroglucinol :Con.HCl 1:1) and mounted with a drop of glycerin-water, characters were observed using a microscope.

d) Determination of Moisture Content:

Five grams of the powdered drug was taken in a weighed flat porcelain dish and was dried in the oven at 100-105o C for five hours andweighed. Repeated the drying and weighing at one-hour interval until difference between two successive weightings corresponds not more than 0.25 %. Constant weight is reached when two consecutive weighing, after drying for 30 minutes and cooling for 30 minutes indesiccators, showed not more than 0.01 g difference

e) Determination of Total Ash

Five grams of powdered Osbeckia muralis Naudin.was taken in a weighed crucible. It was then heated over a burner until all the carbon was burnt off at a temperature not exceeding 4500 C. Then it was cooled in a desiccator and weighed, exhaust the charred mass with hotwater, collect the residue on ash less filter paper, incinerate the residue and filter paper, add the filtrate, evaporate to dry, and ignite and calculated the percentage of total ash with reference to the air-dried sample of the crude drug.

f) Determination of Acid Insoluble Ash

The ash obtained in the previous experiment was boiled with 25 ml of dilute Hydrochloric acid. The solution was filtered using an ash less filter paper. The residue was washed with hot water and ignited in a weighed silica crucible with the filter paper. The weight of thecrucible was measured to get acid insoluble ash

g) Determination of Water Soluble Ash

5 grams of air-dried powder of the test drug was incinerated in a weighed crucible. The ash thus obtained was boiled with 25 ml of distilled water for 5 minutes. The solution was filtered using an ash less filter paper. The residue was washed with warm distilled water and ignited in a weighed silica crucible.

h) Determination of pH value

Operate the pH meter and the electrode system according to the manufacturer's instructions. Standardize the meter and electrodes with 0.05 M Potassium hydrogen phthalate (pH 4.0). solution. At the end of a set of measurements, take a reading of the solution used to standardize the meter and electrodes. This
reading should not differ by more than 0.02 as that of original value with which the apparatus was standardized.Preparation of solutions: A 10 % w/v solution in water filtered through a filter paper when measuring an acid solution or with 0.05 M Sodium Borate when measuring in alkaline

2. **SUCCESSIVE SOVENT EXTRACTION**:

Different solvents like Aqueous, Ethanol, Methanol, Acetone, Chloroform, Petroleum ether, has been used for the extraction of the drug.Two different methods have been adopted. One with Standard procedure using dry drug and other using fresh drug has been followed for the extraction

3. PRELIMARY PHYTOCHEMICAL SCREENING:

Medicinal plants have bioactive compounds which are used to curing various diseases. The extracts were subjected to qualitative phytochemical screening using standard procedure. Phytochemical screening reveals the presence of Alkaloids, Saponins, Tannins, Steroids, Glycosides, Flavanoids etc

4. HPTLC:

One gram of powdered sample was extracted with 10 ml ethanol and kept for cold percolation for 24h and filtered. 5,10 and 15
µl of the above samples were applied on a pre-coated silica gel F_{254} on aluminum plates to a band width of 6 mm using Linomat 5 TLC
applicator. The plate was developed in Toluene: Ethyl acetate (9:1). The developed plates were visualized and scanned under 254 and 366 nm. It was derivatised with vanillin sulphuric acid reagent, visulaised and scanned under 620 nm after heating the plate until the
development of colours. Rf, colour of the spots and densitometric scan were recorded.

5. PHARMACOLOGICAL STUDY

I. TOXICITY STUDIES:

i) Acute Oral Toxicity Study:

Study has been carried out as per OECD guideline425.

General Procedure:

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a) Sample – 5 healthy albino rats of either sex with average weight of 150-200gm will be selected randomly for the study.

b) Design of the study:

Acute toxicological studies of the plant will be done on a sample of 5 Albino rats of either sex weighing 150-200gm. Among those,1 rat will be given 4 times the Kashaya dose orally. After 24hrs of observation if the rat is survived, the remaining rats are subjected for study. The dosage is fixed based on the human adult dose and is converted to animal dose on the basis of body surface area ratio (Paget and Barnes table, 1964). Rats will be observed for seven days for any acute toxicity symptoms.

As per the data observed, statistics will be worked out through the appropriate statistical tests (As per OECD guidelines425 and ClaraMorpugo-et-al 1971).

Gross behaviour was also assessed in all the rats. The procedure involves assessing the observed behavior on a subjective scale awarding-scores on 0-3-point scale as per the average intensity of the phenomena observed. After administering the limit dose i.e. 5ml/kg body weight as specified in the OECD Guidelines 425, the observations were made after every 1 hr. up to 4 hr and then at 24 hr. interval up to 72 hr of drug administration. The rats were placed one by one in the center of three concentric circles (drawn by Chalk on a rubber sheet) of diameter, 7 cm, 9 cm and 13 cm.

Assessment was done for the following parameters: CNS depression, CNS stimulation

IV. ANTIBACTERIAL STUDY

Aqueous extract and Kashaya of Osbeckia muralis Naudin were used to evaluate their antibacterial effect on two organisms viz Staphyllococi aureus (Gram +ve) and Klebsiella pneumoniae (Gram –ve) and compared to standard drug Norfloxacin. Observations were recorded.

A. Method 1: Well diffusion Method

Initially, the stock cultures of bacteria like Staphyllococi aureus and Klebsiella pneumoniae were revived by inoculating in Muller Hinton broth media and grown at 37ºC for 18 hrs. The Muller-Hinton agar plates were prepared and wells were made in the plate.Each plate was inoculated with 18 h old cultures (100 µl, 10-4 cfu) and spread evenly on the plate. After 20 min, the wells were filled with of compound and antibiotic at different concentrations. All the plates were incubated at 37ºC for 24 h and the diameter of inhibition zone were noted.

B. Method 2: Disk diffusion Method

Initially, the stock cultures of bacteria like Staphyllococi aureus and Klebsiella pneumoniae were revived by inoculating in Muller Hinton broth media and grown at 37ºC for 18 hrs. The Muller-Hinton agar plates were prepared. Each plate was inoculated with 18 h old cultures (100 µl, 10-4 cfu) and spread evenly on the plate. After 20 min, the paper disk was made and impregnated with standard anti-bacterial chemical and testing samples (Osbeckia muralis Naudin Kashaya and Aqueous extract) placed in the plate
at different concentrations. All the plates were incubated at 37ºC for 24 h and the diameter of inhibition zone were noted.

V. RESULTS:

I. PHARMACOGNOSTIC STUDY

a) MACROSCOPY:

Morphological features of Osbeckia Muralis are:

Fig 1a.1 Aerial parts

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Fig 1a.2 A twig

Fig 1a.3 Leaves

Leaves	• Leaves are oppositely arranged • Leaves have 3 parallel nerves starting from the base • Elliptic lance-shaped • Length:1-3cm
Flowers	• Funnel shaped • Petals: 4-5 • Colour:Purple-pink
Fruit	• Oblong • 4-ribbed
Flowering	• August-September

Table:1: Macroscopy of Osbeckia Muralis

b) MICROSCOPY:
STEM

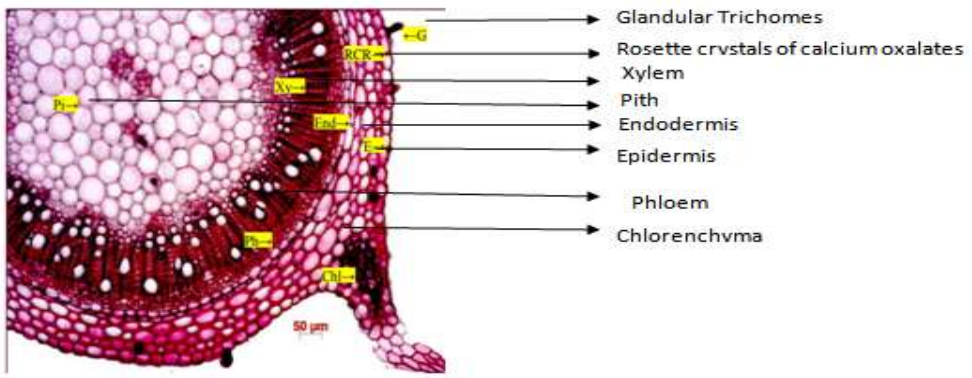

Fig 1b:1: Microscopy of stem of *Osbeckia Muralis*

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Circular in shape with three ridges

- Outer epidermis have glandular trichomes and are multicellular covering trichomes
- multicellular covering trichomes and wins contain chlorenchyma cells
- cortex consist of calcium oxalate crystals
- endodermis covered with one layer of pericyclic fibers
- Centre pith encircles vascular bundlesw, contain xylem and phloem
- Pith composed of parenchymatous cells

LEAF

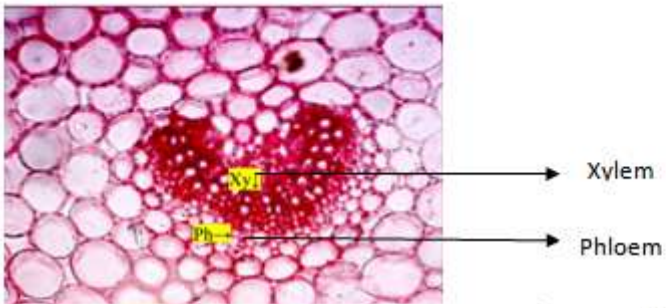

Fig 1b:1: Microscopy of stem of *Osbeckia Muralis*

- Upper epidermis consist of glandular trichomes
- Mesophyll region consist of a layer of compactly arranged parenchyma with in the upper epideremis and loosely arranged spongy parenchyma with in the lower epidermis
- Midrib region contain vascular bundle with xylem and phloem
- Below the vascular bundle, midrib consist of loosely arranged collenchymas cell
- Lamina region also contain calcium oxalate crystals

c) POWDER MICROSCOPY

Reported Powder microscopic features of Osbeckia Muralis are given below:

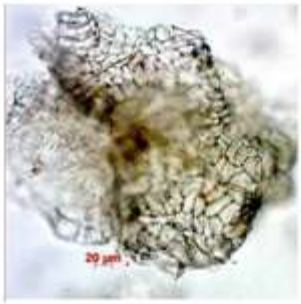

Fig:1c:1:Parenchyma of trichome base

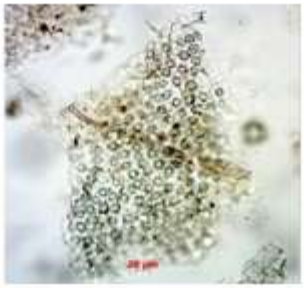

Fig:1c:2:Xylem elements in mesophyll

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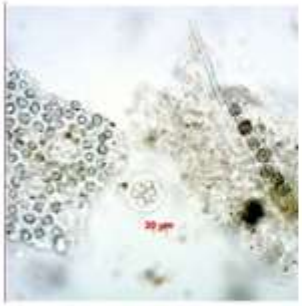

Fig: 1c: 3: Rosette crystal

Fig: 1c: 4: Rosette crystal

Fig: 1c: 5: Vascular

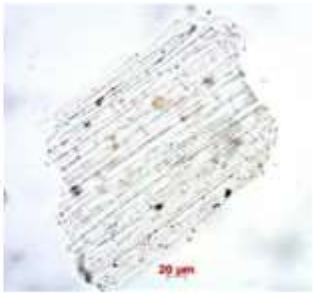

Fig: 1c: 6: Pith parenchyma

Fig: 1c: 7: Upper epidermis

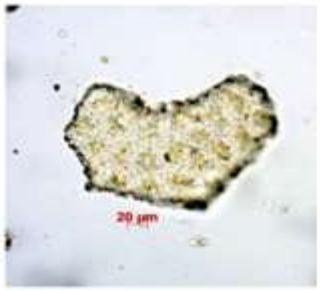

Fig: 1c: 8: Lower epidermis in surface view

Fig: 1c: 9: Multiseriate trichome

Fig: 1c: 10: Glandular trichome

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Fig: 1c:11:Base of a multiseriate trichome

Fig: 1c:12: Apex of a multiseriate trichome

PHYSICO-CHEMICAL SCREENING:

Moisture content	4.4%
Total Ash	9.6%
Acid Insoluble Ash	0.4%
Water soluble ash	0.7%
pH	5.8

Table:2: Physico-chemical properties of *Osbeckia Muralis*

d) ASH ANALYSIS

SL.NO	Components	Observation
1	Carbonates	No colour change
2	Fluorides	No precipitate
3	Chlorides	Curdy white precipitate
4	Sulphates	White precipitate
5	Chromates	No yellow precipitate
6	Phosphates	No precipitate
7	Potassium	Yellow precipitate
8	Sodium	White colour precipitation
9	Aluminium	No gelatinous precipitate
10	Calcium	No white colour precipitation

Table:3: Ash analysis of Osbeckia Muralis

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2) 2) SUCCESSIVE SOLVENT EXTRACTION (PERCENTAGE OF FRESH AND DRY RESIDUE)

Sl. No	Solvent	Percentage of Extract of Osbeckia muralis Naudin (fresh)	Percentage of Extract of Osbeckia muralis Naudin (dry)
1	Water	3.2	14
2	Ethanol	2.2	5.2
3	Methano	2.2	5
4	Chloroform	12.11	2
5	Petroleum ether	2.8	3.3
6	Acetone	3.2	2.4

Table:4: Extraction percentage of Osbeckia Muralis (Fresh and Dry)

3) PHYTOCHEMICAL SCREENING:

a) Determination of active constituents:

SL.NO	Phytoconstituent	Identification test	Observation	Result
1	Alkaaloid	Mayer's Test	Yellow precipitate	P
2	Phenols	Phenol test	No intense colour	N
3	Flavanoids	Shinoda test	Scarlet colour seen	P
4	Carbohydrate test	Benedict's test (Reducing sugar)	Green colour precipitate	P
		Benedict's test (Non-reducing sugar)	Formation of a coloured precipitate	P
5	Proteins	Biuret test	Violet colour precipitate	P
		Millon's test	White ppt formed, turned red on heating	P
6	Tritrepenoides	Liebermann Burchard's test	No Change	N
7	Tannins	Tannin test	A green colour seen	P
8	Saponin	Foam test	Foam seen, but did not persist	N
9	Steroids	Salkowski's test	No colour change	N

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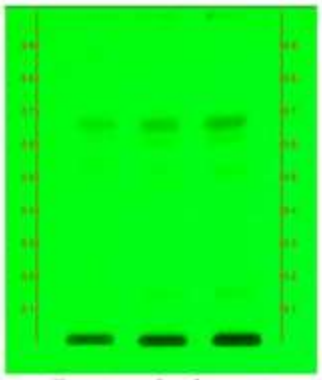
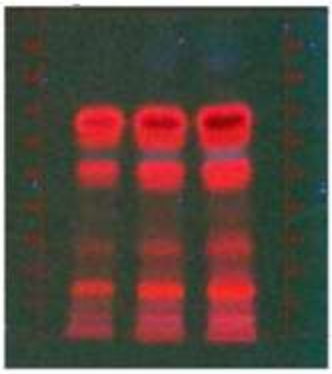
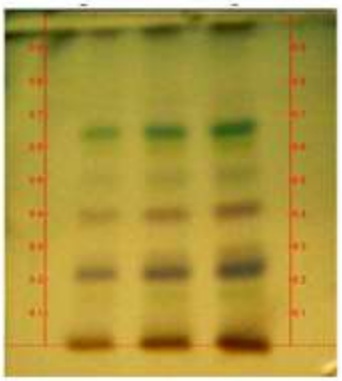
10	Starch		No colour change	N
11	Resin		No change	N

P: Positive **& N:** Negative

Table:5: Phytochemical screening ofOsbeckia Muralis

4) HPTLC

Fig;4a: Under short UV Fig;4b:Under Long UV Fig;4c:Under white light after derivatisation with Vanillin/sulphuric acid

Under short UV	Under long UV	Post derivatization
-	0.05 (F Red)	-
-	0.13 (F Red)	-
0.15 (DL Green)	-	0.15 (Lemon Yellow)
-	-	0.23 (Violet)
-	0.27 (F Red)	-
-	-	0.41 (Purple)
-	0.47 (F Red)	-
0.52(L Green)	0.52(F Red)	0.52 (L Green)
-	0.57 (F Red)	-
0.61(L Green)	-	0.61 (Lemon Yellow)
0.66 (L Green)	0.67 (F Red)	0.66 (D Green)

*L-Light, D-dark, F-Fluorescence

Table:6: HPTLC ofOsbeckia Muralis

5) PHARMACOLOGICAL STUDY

a) Toxicity study

➢ **Acute Oral Toxicity Study**

As the five animals survived without showing any signs of toxicity, the LD50 (Medial Lethal Dose) is greater than the limit dose (5ml/kg) and there were no signs of toxicity or behavioural changes observed when they were observed for the next 14 days.

The Table No.7 contains data related to gross behavioural changes observed during testing. Increased pellet expulsion was observed it indicates possibility of anxiety producing effect. The test

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drug did not produce effect on other parameters studied.

Group	Dose	CNS Depression							CNS Stimulation						
		1hr	2hr	3hr	4hr	24hr	48hr	72hr	1hr	2hr	3hr	4hr	24hr	48hr	72hr
Trial	50ml	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Control	Tap water	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

Table:7:Acute oral toxicity study of Osbeckia Muralis

b) **Anti-bacterial study**:

1) Well diffusion method

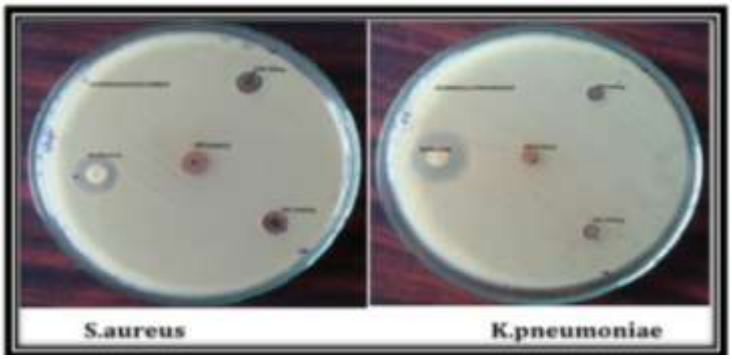

Fig:5:b1:Anti-Bacterial Study (Well Diffusion Method)

Aqueous extract and Kashaya of Osbeckia muralis in different concentrations were subjected to antibacterial study withNorfloxacin as standard drug. Gram +ve and gram –ve strains were used in which Staphyllococi aureus and Klebsiella pneumonia from both strains were selected.

Anti-bacterial study on gram +ve organism (Staphylococci Aureus) Aqueous extract of Osbeckia muralis Naudin showed no inhibitions in 500µg and 1000µg concentrations and Kashaya of Osbeckia muralis also showed no inhibitions in 1000µg concentration.

Anti-bacterial study on gram -ve organism (Klebsiella pneumoniae)

Osbeckia muralis Naudin's Aqueous extract showed no inhibition in 500 µg and 1000µg concentration and Kashaya of Osbeckiamuralis did not show any activity.Datas are given in (Table No.8)

Table:8: Well diffusion anti bacterial study of Osbeckia Muralis

Sample	Staphylococci aureus			Klebsiella pneumonia		
	500µg	1000µg	MIC µg	500µg	1000µg	MIC µg
OM-A	0	0	NF	0	0	NF
OM-K	0	0	NF	0	0	NF
Norfloxacin	34	*	25	38	*	25

* the inhibitions zones were too big to measure
NF- MIC not found

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c) Disk diffusion method

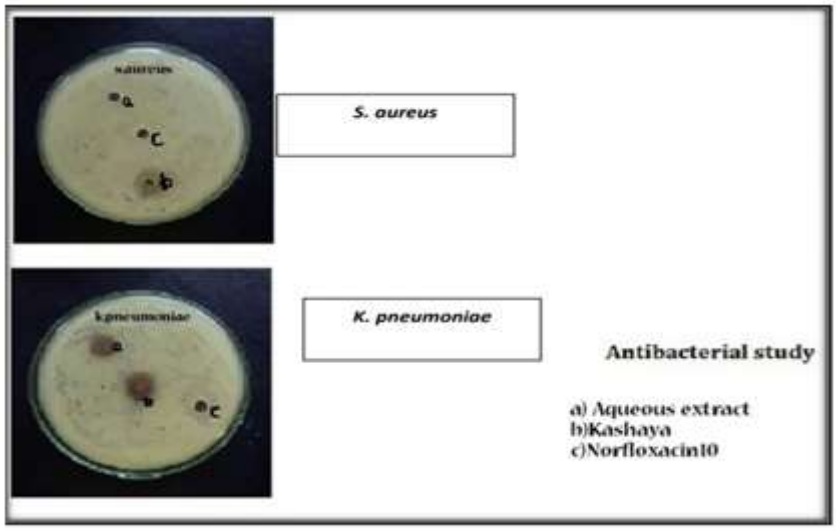

Fig:5:c1: Anti-Bacterial Study (Disk Diffusion Method)

Aqueous extract and Kashaya of Osbeckia muralis were subjected to antibacterial study with Norfloxacin as standard drug. Gram+ve and gram –ve strains were used in which Staphylococci aureus and Klebsiella pneumoniae from both strains were selected.

Anti-bacterial study on gram +ve organism (Staphylococci Aureus)

Aqueous extract and Kashaya of Osbeckia muralis Naudin showed no inhibitions (Table No.9)

Sample	Staphylococci aureus		Klebsiella pneumonia	
	1000µg	MIC µg	1000µg	MIC µg
OM-A	0	NF	12	1000
OM-K	0	NF	24	1000
Norfloxacin 10	30	10	32	10

Table:9: Disk diffusion anti bacterial study of Osbeckia Muralis

VI. CONCLUSION

Osbeckia Muralis belongs to the family Melastomaceae commonly used as ethnomedicine in cough.Through its pharmacognostical study identifying the organoleptic features of the plant. Microscopically the leaves shows glandular trichomes and multicellular covering trichomes. Cortex is having rosette crystals of calcium oxalate.

Phytochemical analysis showed the presence of proteins,carbohydrates,tannins, flavanoids, alkaloids, chlorides,sulphate, potassium and sodium.This will help for the exact identification of the plant for the future reference.

Based on the acute oral toxicological study which showed no signs of toxicity. Antibacterial study with disk diffusion method showed the positive action of Osbeckia Muralis Kashaya in klebseilla pneumoniae.

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