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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL INVESTIGATION OF *MURRAYA KOENIGII*

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ABSTRACT

Medicinal plants are used in herbalism and thought to have some medicinal properties. They form the easily available source for healthcare purposes in rural and tribal areas. Ethanobotany is a distinct branch of natural science dealing with various aspects such as anthropology, archaeology, botany, ecology, economics and medicine, religious, cultural and several other disciplines. Recently, great interest in the above given studies of herbal drugs and traditional remedies is indicated worldwide and there has been an upsurge in the scientific investigations in this area. The Murraya koenigii plant is widely used as herb, spice, condiments and also used to treat various types of ailments in Indian traditional system. World's about 80% population relies upon herbal products, because they have been considered as safe, effective and economical. The present study was aimed to review the ethanobotanical properties, pharmacognostic, phytochemical and pharmacological properties With anti repellent properties itself in of *Murraya koenigii* plant. The various parts of this plant are widely used by different tribal communities. The leaves of plant are use as tonic, stomachic, carminative, internally in dysentery, vomiting. Used as antihelminthic, analgesic, cures piles, allays heat of the body, thirst, inflammation and itching. Following various claims for cure of numerous diseases, efforts have been made by researchers to verify the efficacy of the plant through scientific biological screening. A scrutiny of literature reveals some notable pharmacological activities of the plant such as activity on heart, anti diabetic and cholesterol reducing property, antimicrobial activity, antiulcer activity, antioxidative property, cytotoxic activity, anti diarrhea activity, phagocytic activity and many more medicinal values.

KEYWORDS: Curry leaf, ethanobotany, pharmacognosy, pharmacology, phytochemistry.

1. INTRODUCTION

India is frequently known by enormous biodiversity of medicinal plants. Among them murraya Koenigii have a lots of bioactive principles due to which plant has been proven as the medicinally important plant but least or no attention received by the scientist. Murraya Koenigii is proven as the natural medicinal plant.[1] There are different forms of murraya Koenigii due to which they are found as the useful plant such as extract, essential oil. Vegetable with many uses. It is belonging to family rutaceace India is rich in the medicinal herbs and therefore, it can be accurately called the "botanical garden of the world" medicinal plants have been used by mankind for its curative quality since the starting of human civilization. The history of curry leaves are In Tamil and Kannada literature it was updated as word kari and spiced sauce.

The species name given by the botanist Johann Konig. The genus Murraya commemorates Swedish physician and botanist Johann Andreas Murraya who died in 1791. Hence the botanical name of the curry leaves is Murraya Koenigii (Linn) spreng belong to family Rutaceace We have been getting a huge amount of medicinal agents since a long time from nature and we can produce multitude of modern drugs with the help of these agents. The basic medicinal property of these plants lies in some chemical substances. These chemical substances produce a definite physiological action on human body which is generally known as phytochemicals. These chemicals are nonnutritive and act like shield against diseases. Murraya Koenigii is widely use in Indian cookery for centuries and have a versatile role to play in traditional medicine industry. Herbal medicine has proved efficacious and potent in the treatment of many chronic diseases that orthodox medicine cannot cure. India has rich plant diversity and houses about 47,000 plant species, out of these 7,500 have medicinal value; but only 800 plant species are used in the preparation of herbal drugs. Alarge number of plants still remain unexplored with regard to their medicinal properties and they can be sources of potential bioactive compounds for the development of new "leads" to combat various diseases. The present review is on Murraya Koenigii which is commonly called as "curry leaf" in English and meetha neem in Hindi. It is an important medicinal plant of our country and is grown in almost every house for its aromatic leaves.

Scientific studies have been conducted regarding the efficacy of different plant parts in the treatment of various diseases. There is a need to review the information available in literature

on Murraya phytochemical studies, so that it would aid future research by phytochemists, pharmacologists, clinic.[1,2]

1.1 Drug profile

Taxonomic status[12]

a. Kingdom –Plantaes

b. Sub-kingdom –Tracheobionta

c. Superdivision–Spermatophyta

d. Division - Magnoliophyta

e. Class - Magnoliospida

f. Subclass – Rosidae

g. Order –Sapindales

h. Family – Rutaceae

i. Genus - Murraya J. Koenigii

Fig no 1

1.2 Synonyms

1.2.1 Synonym in Indian Language

Curry Leaf (English), Karepaku (Andhra Pradesh), Narasingha (Assam); Barsanga, Kartaphulli (Bengal); Gorenimb (Gujrat); Mitha Neem (Himachal Pradesh); Kathnim, Mitha Neem, Kurry Patta (Hindi); Karibeva (Karnataka); Kariveppilei (Kerala); Gandhela, Gandla, Gani (Kumaon); Bhursanga (Orissa); Mahanimb (Sanskrit); Karivempu (Tamilnadu).

1.2.2 Synonym in other language

Burmese :Pindosine; Danis h : Karrry bald; Dutch : Kerriebladeren; English : Curry leaves; French :Feuilles de curyy German : Curryblatter; Indonesian : Daunkari; Italian :Fogli de Cari; Spanish : Hoja.

1.3 Biological source[1]

The species name commemorates the botanist Johann Konig. The genus Murraya commemorates Swedish physician and botanist Johann Andreas Murraya who died in 1791. Hence the botanical name of the curry leaves is Murraya Koenigii. The botanical name of the curry leaves is Murraya Koenigii (Linn)spreng belong to family Rutaceace.

1.4 History

The history of curry leaves is seen in early 1st to 4th century AD. In Tamil and Kannada literature it was updated as word 'kari' with its uses. The word now popularly used for the murraya Koenigii is curry leaf which is originated from Tamil word Kari which means as 'spiced sauce'. In the early literatures of Tamil and Kannada the use of murraya Koenigii is described as the flavouring agent for the vegetables. Today murraya Koenigii are grown as the cultivated crop in India, Sri Lanka, Southeast Asia, Australia, Pacific Islands and Africa as flavouring agent for the food.

1.5 Geographical source[5,3]

Murraya Koenigii originates from east and south part of India, Pakistan, Sri Lanka, China and Hainan but widely cultivated in South-East Asia and some parts of the United States and Australia. It grows throughout India up to the height of 1500 to 1655 m from sea level and in the Andaman Islands. It is also available in other part of Asian region like in moist forests of 500-1600 m height in Guangdong, Shainan, S Yunnan (Xishuangbanna), Bhutan, Laos, Nepal, Pakistan, Sri Lanka, Thailand, Vietnam. Together with South Indian immigrants, curry leaves reached Malaysia South Africa and Reunion Island Out of the 14 global species that belong to the genus Murraya, only two are known to be found in India, which is Murraya Koenigii (Spreng) and Murrayapaniculata(Jack) Can grow in full sun or light shade. Murraya Koenigii is distributed from south and East Asia to Australia.

1.6 Chemical Constituent

Murraya Koenigii is very rich source of organic compounds with different chemical composition such as alkaloids, flavonoids carbohydrates, and sterol is present in the plant extract prepared in solvents such as petroleum ether, ethyl acetate, chloroform, ethanol and water. The major chemical constituents are explained for the confirmation of the phyto-constituents in the plant extract, various numbers of tests were performed: Essential oil: Tender leaves contain 0.8% oil as obtained by steam distillation. A number of reports are there on the essential oil composition of leaves obtained by steam distillation, solvent extraction or by fluid carbon dioxide extraction. The oil composition shows phenotypic and genetic variability in diverse origin germplasm lines of curry neem 80-87. The chemical composition of the essential oil from leaves of M. Koenigii varies with variation in agrclimatic and geographical variation. The leaves oil of murraya Koenigii from Southern Nigeria88 contains sesquiterpenes (89.1%). The major constituents were b-caryophyllene

(20.5%), bicyclogermacrene (9.9%), a-cadinol (7.3%), caryophyllene epoxide (6.4%), b-selinene (6.2%) and humulene (5.0%). The fresh leaves of murraya Koenigii from Dehradun89 contains apinene (51.7%), sabinene (10.5%), ß-pentene (9.8%), ß- caryophyllene (5.5%), limonene (5.4%), bornyl acetate (1.8%), terpinen-4-ol (1.3%), g-terpinene (1.2%) and a-humulene (1.2%) as the major constituents. The essential oil of leaves consists mainly of monoterpenoids and its oxygenated derivatives84. The major oil constituents are β-caryophyllene (35.8%), βphellendrene (2.57%), α- pentene (0.26%), β-elemene (0.18%) and β thujene (4.12%) as determined by GC- MS of steam distillate. Other components are α-caryopyllene (9.17%), cardinene (8.43%), selinene (8.88), linalool (0.27%), trans ocimene (3.12%), gujunene (1.46%)80. Volatile oil obtained form flowers consists of 34.4% monoterpenoids and 43.9% of sesquiterpenoids. The major components are βcaryophyllene (24.2%), (E)- β-Ocimene (18.0%) and linalool (8.0%)90. Volatile oil composition of the fruit of M. Koenigii has been first time reported by Awasthi et.al. As per their studies hydrodistillation of fruits of murraya Koenigii resulted in the isolation of 0.13% of oils (w/v) on fresh weight basis respectively. GC and GC-MS analysis resulted in the identification of 73 constituents comprising 98.8% of the oil, of which the major ones were caryophyllene oxide (10.3%), b-caryophyllene (8.5%), tridecanoic acid (8.2%), dehydroaromadendrene (8.0%), terpinen-4-ol (8.0%), a-cadinol (7.3%), and (Z,E)-farnesol (5.7%).91N OCH3.

1.7 USES

1.7.1 Antifungal activity

The essential oil from leaves of Murraya Koenigii showed antifungal activity against C. albicans, C. tropicalis, A. nigerA. fumigates, at a dilution of 1:500. The ethanolic extract of the leaves showed fungi toxicity against Colletotrichum falcatum and Rhizoctoniasolani. The ethanolic extract of the roots and also the whole plant excluding roots Murraya Koenigii, however, did not show any antifungal activity against Cryptococcus neoformans, Trichophytonmentagrophytes and Microsporumicans.[8]

1.7.2. Antibacterial activity

The essential oil from Murraya Koenigii leaves showed antibacterial effect against B. subtilis, S.aureus, C. pyogenes, P. vulgaris and Pasteurellamultocida. The pureoil was active against the first threeorganisms even at a dilution of 1: 500. Theacetone extract of the fresh leaves of Murraya Koenigii on fractionation give three bioactive carbazole alkaloids named as

mahanimbine, murrayanol and mahanine, which has shown mosquitocidal, antimicrobial and topoisomerase I and II inhibition activities.

1.7.3. Antiprotozoal activity

extracts (55%) of Murraya Koenigii whole plant excluding roots (extract A) and roots alone (extract B) were screened for their pharmacological actions. Extract A showed antiprotozoal action against Ent. Histolytica, antispasmodic effect showed antiprotozoal action against Ent. Histolytica, antispasmodic effect on isolated guinea pig ileum, whereas extract B showed antiprotozoal activity against Ent. Histolytica and as well as antihypertensive.

1.7.4. Antidiabetic property

Mahanimbine a chemical constituent of M. Koenigii was isolated from column chromatography of the petroleum ether extract of dried plant. The anti-diabetic activity was performed on the streptrozotocin induced wistar rats by using pure compound at a dose of 50 mg/kg and 100 mg/kg. The possible mechanism by which the mahanimbine decreases blood sugar level may be by potentiating of insulin effect either by increasing the pancreatic secretion of insulin.

1.7.5. Hypocholesterolemic activity

Hypocholesterolemic activity was checked in aged mice, which was done by using crude ethanol extract of plant leaves of M. Koenigii. The experiment was confirmed by observing a decrease in cholesterol level in dose dependent manner in aged mice. The dose of 500 mg/kg was found more efficient than the 300 mg/kg and was comparable with the standard cholesterol reducing agent, Simvastatin.

1.7.6. Antiulcer Activity

Antiulcer activity of aqueous and ether extracts of M. Koenigii was studied in reserpine induced gastric ulcer model in albino rats. Extracts were effective in gastric ulceration and suggested as protective as ranitidine. Crude aqueous extract of leaves showed antiulcer activity.

1.7.7. Anti Diarrheal activity

The bioassay guided fractionation of the n-hexane extract of the seeds of M. Koenigii resulted in the isolation of three pure compounds of bioactive carbazole alkaloids, kurryam, koenimbine and koenine. Of the three compounds kurryam and koenimbine exhibited

significant inhibitory activity against castor oil-induced diarrhea and PGE2-induced enter pooling in rats. The compounds also produced a significant reduction in gastro-intestinal motility in the charcoal meal test.

1.7.8. Analgesic and antinociceptive activity

The methanolic extract of leaves showed analgesic effect in hot plate model and formalin induced paw licking response in mice. The activity might be linked to the processes involved in the prevention of sensitization of nociceptors, down regulation of the sensitized nociceptors or blockade of the nociceptors at peripheral and central levels. Methanol extracts were taken at different concentrations.8

1.7.9. Wound Healing effect

Male albino rates were used to check the wound healing activity by screening with ethanolic extract of leaves of M. Koenigii. In the excision, wound healing model reveals that three groups which were taken for wound healing activity.

1.7.10. Anti-lipid peroxidative activity

The status of lipid peroxidation was investigated in rats fed with M. Koenigii. The concentration of melondialdehyde showed a significant decrease, while hydroperoxides and conjugated dienes were significantly increased in liver and heart. Glutathione levels in liver.8

2. MATERIAL AND METHOD

2.1. Material

2.1.1 Collection of plant material

Fresh leaves of murrayaya Koenigii collected from manewada, nagpur, maharashtra. during the month of march The collected leaves were identified taxonomically and authenticated by Mr. Dorle Taxonomist, Department of Plant biology & biotechnology and department of pharmaceutical sciences in faculty of medicine in Rashtrasant Tukadoji Maharaj Nagpur University, (RTMNU) Nagpur. A voucher specimen (No: 10141) was deposited at Herbarium, Department of Plant Biology in pharmaceutical department. The leaves were washed thoroughly 2-3 times with running tap water and once with sterile water, air dried, powdered sterile water, air dried powdered and used for extraction. Asoaked for 10 minutes in sterilized distilled water and then air dried. The dried leaves were ground to fine powder with the help of pestle and mortar. The plant powder was stored in air- tight bottles. Aqueous extract 20% w/v and alcoholic extracts 10% w/v were prepared for use in the study.

2.1.2 Selection of bacterial strain

Medically important bacterial strain used in this study were staphylococcus aureus lacto bacilli, and enterobacterarogenes.

2.1.3 Standard of refererance antibiotic

Ampicilline, streptomycine.

2.1.4 Chemicals

Ethanol, sodium hydroxide, hydrochloric acid, sulphuric acid, phloroglucinol, mayers reagent, benedict reagent hagers reagent, wagnaer reagent, dragondroffreagent. biuret reagent, ninhydrin reagent acetic acid, 5% ferric chloride reagent, potassium dichromate, fehling solution A and fehling solution B, streptomycin, Ampicilline.

2.1.5 Equipments

Hot air oven, autoclave, incubator, mufflefurnance, dessicator, rotary evaporator.

All material used are of A grade quality.

2.2. Method

2.2.1 EXTRACTION OF PLANT[2,5,8]

Plant extract of ***Murraya Koenigii*** was extracted by various method depend on which part of plant extract to be used mainly there are various method used for extraction like hydro distillation, steam distillation, maceration, hot diffusion method, The most suitable method used for extraction of volatile oil is hydro distillation or steam distillation give highest yield and with the use Clevenger's apparatus.

In these process we follow two method

- Maceration method
- Hot infusion method

i) Maceration method

The plant material (leaves) were shade dried and powdered by mechanical grinder. The dried powder then extracted with ethanol to give there extract respectively. The plant material (leaves) 10 gm sequentially extracted with ethanol (150ml) by maceration. Process should be take place for about 5days at room temperature. The obtained extracts were filtered by using what man no. 1 filter paper. The extract were evaporated to obtain syrupy solution.

ii) Infusion Method

Infusion method is process of extracting chemical compound or flavor from plant material in a solvent such as water, oil, alcohol. Allow material to be suspended in solvent over time the process known as steeping. There are mainly two types of infusion methods.

(a) Cold infusion

These method is ideal for slimy herb and herb with delicate essential oil but major disadvantage of these method is that they will not provide effective yield as compare to hot infusion method.

(b) Hot infusion: These method give effective yield while these method is not suitable for the heat labile drug in which they become deterioration because of direct heating.

3. PHARMACOGNOSTIC

3.1 Morphological characteristics

- Colour: green
- Odor: Pleasant
- Taste : Sweetish

3.2 Microscopical characteristic

Upper and lower epidermis composed of rectangular arrange call cover with cutical Uniserate covering tricomes globule elongated warty cystolith extending from epidermis.

3.4 Total Ash Value[10,11]

Take an empty crucible. Ignite empty crucible until red hot. Further weigh the crucible then add 2g of sample to it and weigh again then place it in muffle furnace at 600^0c± 25ºC to complete incineration to get whitish residue removecrucible from furnace and weigh again by using formula calculate total ash.

FORMULA: Wt. of empty crucible=X, Wt of drug taken=Y, Wt. of empty crucible +Ash= Z

Wt of Ash=(Z-X)

Total ash value of sample= 10 0(Z-X)/Y%

3.5 Sulphated Ash Value

Take an empty crucible. Ignite empty crucible until red hot. Further weigh the crucible then add 2g of sample to it then gently ignite to completely charred and weigh again then place it

in muffle furnace at 800 whitish residue remove crucible from furnace allow to cool repeat the procedure with few drops of sulphuric acid to get constant results.

3.6 Moisture Content

Take 5 g of powdered drug and transfer it to a premeasured petridish and place it in oven at 104ºC. Then remove and weigh the petridish from oven, take consecutive three reading of compound. Difference between initial reading and the final reading is measured, this is done to calculate percent loss on drying.

Formula: Initial weight –final weight/initial weight*100.

4. PHYTOCHEMICAL EVALUATION

4.1. TEST FOR CARBOHYDRATE[10,11]

4.1.1 Fehling test: Take sample of about 2ml +add 0.5ml of fehling solution A+..5 ml of fehling solution B heat and observe brick red precipitation colour represented the the amount of sugar present.

4.1.2 Benedict test: Mix equal volume of test solution with benedict reagent Boil in waterbath for 5min solution appear green yellow colour depend upon amount of reducing sugsr present in that compound For presence of reducing sugar in that compound.

4.2. TEST FOR AMINO ACID

4.2.1 Ninhydrin test: Take 3ml of test sample then add 3 drops of 5% ninhydrin solution to the test tube. Place the test tube over the boiling water bath for 10 min. Appearance bluish purple colour indicated that amino acid might be present.

4.3. DETECTION OF ALKALOIDS

4.3.1 Mayer's test: Test substances were dissolved in a few drops of 2N hydrochloric acid. The contents were shaken and the aqueous layer was decanted followed by addition one or two drops of Mayer's reagent to the residue. Appearance of white turbidity or precipitate indicated the presence of alkaloids.

4.4. TEST FOR FLAVANOIDS

4.4.1 Shinoda Test: Take dry powdered extract with 5ml of 99% ethanol add few drops of Hcl0.5gm magnesium turning added to the ethanolic extract orange red pink colour appears indicate the presence of flavanoids.

4.5. DETECTION OF TANNINS

Test material was mixed with one or two drops of basic lead acetate solution. Formation of white precipitate indicated the presence of tannins.

4.6. DETECTION OF SAPONINS

4.6.1 Foam test: The test material was mixed with a few drops of water and shaken well. Foamy lather formation indicated the presence of saponins.

4.7. DETECTION OF QUINONES

A few drops of sodium hydroxide were added to the plant material. Formation of blue green or red colour indicated the presence of quinones.

4.8. DETECTION OF GUM

One or two drops of water was added to the test material and shaken well. Formation of swells or adhesive indicated the presence of gum.

5. ANTIMICROBIAL ASSAY

The disc diffusion method was followed for determining inhibitory activity of the plant extracts on the specified bacterial isolates using Muller Hinton Agar plates. The medium was sterilized by autoclaving at 121°C for 15 minutes, poured in petriplates, allowed to solidify at room temperature, incubated at 37°C overnight. The sterilized cotton swabs dipped in bacterial inoculum were swabbed over agar plates in order to spread the inoculum uniformly. After drying for five minutes, sterile discs dipped in 10µl of the plant extracts were placed on the surface of the inoculated medium and the extracts were then allowed to diffuse for 5 minutes. The plates were incubated at 37°C for 24-48 hours and observed for the inhibition of the growth around the discs thereafter. The antibacterial activity was determined by measuring the diameter of zones of inhibition of bacterial growth around the discs. Ampicillin (10mcg) and Gentamicin (10mcg) were also included in the assay against Staphylococcus aureus.[1,4]

REPELLANCY TEST

The repellence of the volatile oil was evaluated using the instrument that has been modified from Y-tube Olfactometer. This instrument was made from a transparent prospex and has been fabricated with a shape of Y. The test has been done by using Blat aria (5 in number). The observation during the test was recorded. The percentage of repellency was calculated based on the Formula: % Repellence = [(C-T)/Cx100] Where, C is the total of Blat aria that

landed on the control T is the number of Blat aria that landed on the treated area of the essential oil From Table-1 the present of small amount of α-pentene and Caryophyllene indicates that the essential oil of murraya Koenigii leaves has a potential to be used as the active ingredients for natural based insect repellent. The repellency test was conducted using 5 Blat aria (in number) to prove feasibility of the essential oil of Murraya Koenigii to repel insect. The test was performed using Y shaped transparent prospex as shown in Figure-4 that was a modification from Y-tube Olfactometer.[2]

6. RESULT AND DISCUSION

6.1 Pharmacognostic evaluation

Table 1: Pharmocognostic evaluation of *Murraya Koenigii.*

Evaluation	Observation
Colour	greenish
Odour	Pleasant
Taste	sweetish
Shape	Lanceolate

The morphological activity of ***Murraya Koenigii*** was studied it was evaluated that the color of fresh found to be greenish and having a pleasant odor and shape of lanceolate of sweetish taste.

Fig no 2

6.2. Microscopical characteristic

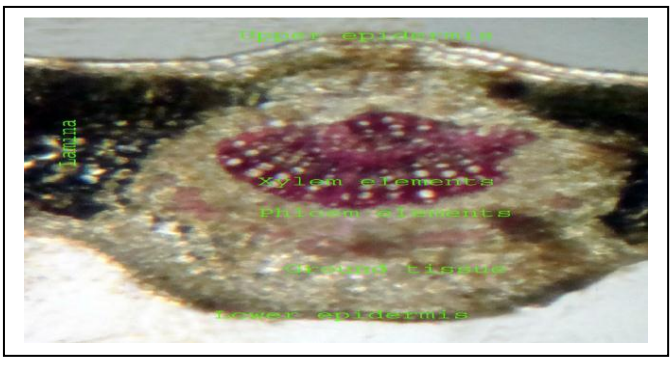

Fig no 3

Upper and lower epidermis composed of rectangular arrange call cover with cutical. Uniserate covering tricomes. Four cell grandular trichome Layer of palisade cell contain oil globule elongated warty cystolith extending from epidermis.

6.3 PHARMOCOGNOSTIC EVALUATION OF *MURRAYA KOENIGII*

Table 2: Pharmocognostic evaluation of *Murraya Koenigii.*

Sr.no	EVALUATION	TEST	STANDARD
1	Total Ash Value	7.9%	8.9%
2	Sulphtated Ash Value	6.5%	7.025%
2	Moisture	10%	15%

Total ash value of Murraya Koenigii was found to be 7.9%, while the standard value of total ash will 8.9. The sulphated ash value was found to be 6.5% and 7.25% The moisture contain of test sample was found to be 10% and that of standard is 15%.

6.4: PHYTOCHEMICAL EVALUATION

Table 3: Phytochemcal evaluation of *Murraya Koenigii.*

PHYTOCHEMICAL	RESULT
Carbohydrate	+++
Amino Acid	+++
Saponin	---
Alkaloids	+++
Tannin & phenolic compounds	+++

The phytochemical evaluation of ethanolic extract shows following result in extract. The presence of carbohydrate, amino acid, alkaloid and tannins with some amount of phenolic compound with absence of saponin extract.

6.5 ANTIMICROBIAL ASSAY

The test sample extract of *Murraya konigii* form the zone of inhibition of about 8mm in the dose of 10mg/ ml but that of standard was found to be 23mm. While that of ampicillin drug of reference standard forms the zone of inhibition of about 14mm in dose of 1 0mg/ml. While that of standard is 14 to 16mm.

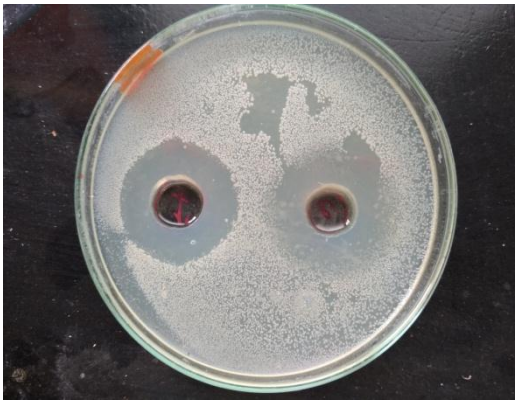

Fig 4: Petridish with zone of inhibition.

DRUG	DOSE	ZONE OF INHIBITION	Standard
Ampicillin	10mg/ml	14mm	14- 16mm
Murraya Koenigii	10mg /ml	8mm	23m m

7. CONCLUSION

The result shows the methanolic extract of *Murraya Koenigii* exhibited significance of antimicrobial activity on staphylococcus aureus and could be a good candidate for antimicrobial drugs. Also study reveals that pharmacognostic, phytochemical study was comparatively same as that of standard value. So being non toxic, economical and easy available and due to herbal nature of Murraya Koenigii have more preference over synthetic drug.

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