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ANTIBACTERIAL ACTIVITY OF METHANOLIC EXTRACT OF MITRAGYANA PARVIFOLIA (ROXB) KORTH

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ABSTRACT

The Antibacterial Activity of methanolic extract of Mitragynaparvifolia(Roxb) *korth* was investigated against some human pathogenic microorganisms *(staphylococcus aureus*and *E.coli).*The tested extracts showed very strong antibacterial activity against all organisms. The antibacterial activity was evaluated by measuring the zone of inhibition using cup plate method. The strongest inhibition activity of the leaves of methanolic extract was observed against staphylococcus aureusand E.coli. This study also investigated the role of environmental factors on the antimicrobial activity of this plant.

KEYWORDS: Antibacterial activity, Soxhlet extraction, Ultrasonicextraction, well diffusion method.

INTRODUCTION[1,2,4,5]

Antibacterial activity is the study of bacteria. For the study treatment of diseases inhibitory chemicals employer to kill the bacteria and to prevent the growth of bacteria called antibacterial agent. Medicinal plants represents the rich source of antibacterial agents. The development and spread of resistance to the existing antibiotics by bacteria are due to indiscriminate use of commercial antibacterial drugs commonly used in the treatment of various diseases. Although a number of plants with antibacterial activities have been identified, great number of still remains unidentified.

Mitragynaparvifolia (Roxb) *korth* belonging to family Rubiaceae is commonly known as Kaim. The plant grows throughout India, in deciduous and evergreen forests. The chemical constituents of the plant are pyroligneous acid, methyl acetate, ketones and aldehydes. The plant is credited with innumerable medicinal properties and is widely used by tribal people and other ayurvedic practitioners. The bark and roots are used to treat fever, colic, muscular pain, burning sensation, poisoning, gynecological disorders, cough, edema and as aphrodisiac. The fruit juice augments the quantities of breast milk in lactating mothers and also work as lacto depurant. Wounds and ulcers are dressed with its leaves to alleviate pain, swelling and for better healing. After extensive literature survey it has been found that, though the plant has great potential for its antibacterial activity, at best of our knowledge nobody has yet documented this activity on leaves So, in this study we have attempted to investigate the antibacterial activity of the leaves.

Name of plant: MitragyanaParvifolia(Roxb) korth.

Biological Source: It consist of dried leaves of *mitragynaparvifolia (Roxb) korth*belonging to family Rubiacae.

Fig. 1: Whole Plant

Fig. 2: Leaves

Plant Profile

Scientific classification	
Kingdom:	Plantae
(unranked):	Angiosperms
(unranked):	Eudicots
(unranked):	Asterids
Order:	Gentianales
Family:	Rubiacae
Genus:	*Mitragyna*
Species:	*MitragyanaParvifolia*(Roxb) korth
Binomial name	
MitragyanaParvifolia(Roxb) korth	
Synonyms	
StephegyneparvifoliaKorth. Kaim (Eng) Kaddam(Hindi), Kongu(Kan),	

Chemical Constituents[1,2]

Pyroligneousacid, Methyl acetate, ketones, aldehde, Mitraphylline, Isomitraphylline, Pteropodine, oxindolic alkaloids.

NEED OF PRESENT INVESTIGATION

In the present investigation an attempt is made to test the antibacterial activity of Methanolicextract of leaves of Mitragynaparvifolia(Roxb) korth by using the disc diffusion method.

AIM AND OBJECTIVES

To perform Antibacterial activity and phytochemical screening of Mitragyana Parvifolia (Roxb) korth. The objectives of my present investigation were, To authenticate the *Mitragyana Parvifolia*(Roxb) korth plant, To extract out the leaves of *MitragyanaParvifolia*(Roxb) korth by ultrasonication extractionmethod, To check the Phytochemical Constituent of the extract, To check the Antibacterial activity of extract by using Cup-plate method.

PLAN OF WORK

It includes following steps such as Selection of plant, Collection of plant, Authentication plant, Extraction of plant by using ultrasonication extraction method with methanol solvent,

Phytochemical test of extract, To check antibacterial activity of plant extract.Theequipments required were IncubatorBTI-25, Amp-15Hot air ovenLc-TAutoclaveLab-hosp-236|084.

MATERIAL AND METHOD

A. Selection of plant

The fresh leaves of the plant will be collected from area of Yashoda Technical Campus, Wadhephata, Satara, Dist. Satara, Maharashtra.

B. Authentication of Plant

The Plant will be authenticated head of department of Yashvantrao Chavan institute of sciences, Satara.

C. Preparation of *MitragyanaParvifolia*(Roxb)korth Extracts[7,8,9]

Principle

Sonication is the act of applying sound energy to agitate particles in a sample, for various purposes. Ultrasonic frequencies (>20 kHz) are usually used, leading to the process also being known as ultrasonicationor ultra-sonication.

Procedure

Take 5 gm of drug powder in a beaker & add 50 ml of methanol.Then kept this beaker in sonicator bath for a 15 min, 30 min, 45 min. respectively.After the completion filter the extract in petri dish & it allow to drying or evaporation.After this calculate the % yield.

Cultures Used

E. coli, and Staphylococcus aureus were collected from theYashvantraoChavan institute of sciences, Satara.Department of biotechnology.

Culture Media

Nutrient agar medium (for *E.coil*) and a dextrose agar medium (for *Staphylococcus*) were these microorganisms in this study.

Antibacterial Activity

The antibacterial activity studies were carried out by using cup plate method. The nutrient agar media was sterilized at 121 °C under 15 psi pressures for 30 minutes. After cooling to about 65 ^{0}C, 25 ml of the medium was poured in Petri-dish. The plates kept at room temperature for solidification and stored at 4 ^{0}C until using. The same process was applied

with dextrose agar plates which were used for the growth of Staphylococcus Bacterial culture was spread over the nutrient agar plates by using separate sterile spreader. Holes were made in the medium by using 7 mm corn borer. The dried plant extract was dissolved in methanol to final extract of 100 mg/ml. Each hole in plate was filled with 2ml of plant extract. Methanol was used as a negative control in one of the plates. The plates were incubated for 24-48 hours at 37°C along with negative controls. The antibacterial activity of each extract was recorded based on the inhibition of bacterial growth by the extract at the end of incubation period. At the end of the incubation period the zones of inhibitions were measured to the nearest millimeter. The inhibition zone is the area surrounded the hole and there is no growth of inoculated microorganism. For confirmation of the results each test was performed in duplicate.

Cup plate method use for Antibacterial Activity[7]

All the sterilized materials were kept in the aseptic area. Bacterial suspensions (3ml) were then poured in the plates. As soon as nutrient agar attained 50^0c temperature, 20ml of media was poured in to the Petri plates containing bacterial suspension and plates were rotated to mix the suspension with media. When the agar got solidified bores were made in the plate with sterile borer of 8mm diameter. In each plate six bores were made. out of which one is meant for addition of standard, two for negative control of blank solvent of standard and blank and remaining three bores for addition of same concentrations of sample. 100 micron liter of sample was added in each cylinder. The plates were kept to allow diffusion at room temperature for bacteria; sabouraud dextrose agar was poured in the petri plate, allowed to solidify. Bacterial suspension was then sprayed uniformly over the surface of agar. All the procedure was same as that of antibacterial activity. Bacteria were incubated at room temperature for 48h. The diameter of zone of inhibition was accurately measured by zone reader in each treated plate and was compared with standard at tested concentration.

Extraction of leaves of MitragyanaParvifolia(Roxb) korth

Observation Tables

Observation	For 15 min	For 30 min	For 45 min
Colour	Green	Green	Green
Odour	Characteristic	Characteristic	Characteristic
Taste	Bitter	Bitter	Bitter

15 min

1. Wt. of dry crude drug (powder): 5gm.

2. Wt. of empty petri plate: 41.38 gm.
3. Wt. of extract with petri plate: 42.50gm.
4. Wt. of extract: 1.12gm.

30 min

1. Wt. of dry crude drug (powder): 5gm.
2. Wt. of empty petri plate: 40.22gm.
3. Wt. of extract with petri plate: 42.30gm.
4. Wt. of extract: 2.08gm.

45 min

1. Wt. of dry crude drug (powder): 5gm.
2. Wt. of empty petri plate: 40.29 gm.
3. Wt. of extract with petri plate : 41.85gm.
4. Wt. of extract: 1.56 gm.

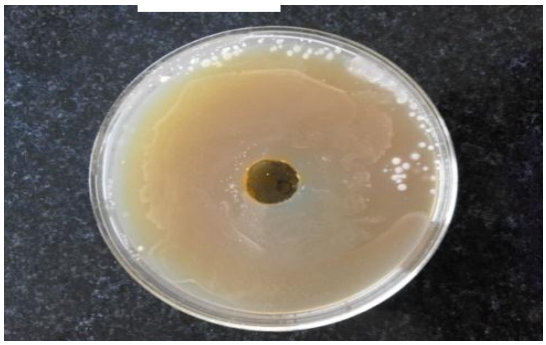
Phytochemical screening[6,8]

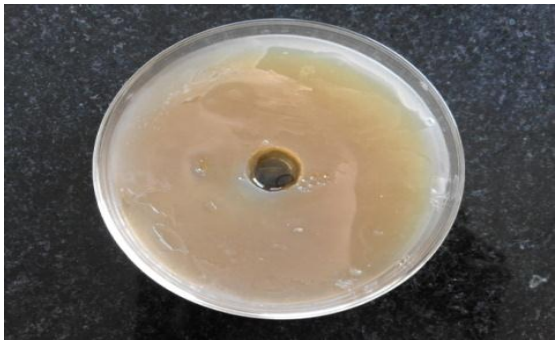
Sr.	Tests	Leaf extract	Leaf extract	Leaf extract
1	**Alkaloids**			
	Dragendorff's test	+	+	+
	Wagner's test	+	+	+
	Mayer's test	+	+	+
	Hager's test	+	+	+
2	**Steroids**			
	Libermann-buchard test	+	+	+
	Salkowaski test	+	+	+
3	**Tannins**			
	Lead acetate solution	+	+	+
	Bromine water	-	_	_
	Acetic acid solution	-	_	_
	Dil.Potassiumpermagnate	+	+	+
4	**Test for saponins**			
	Foam test	+	+	+
5	**Test for flavonoids**			
	Lead acetate test	+	+	+

Observation of Antibacterial Activity

Staphylococcus aureus

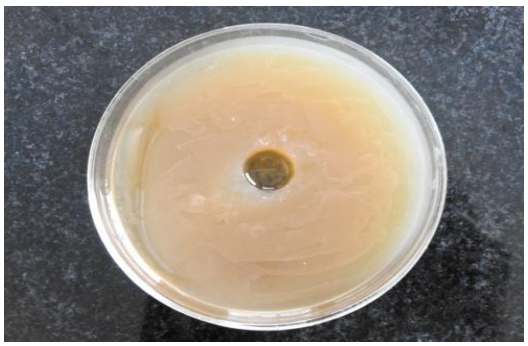
Standard

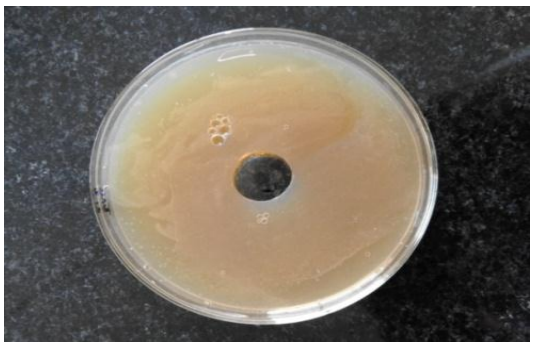




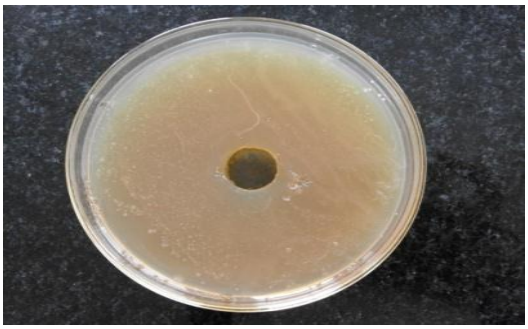
Sr. no.	Concentration of extract	Average zone of inhibition in mm
1.	20µg/ml	28mm
2.	100µg/ml	32mm

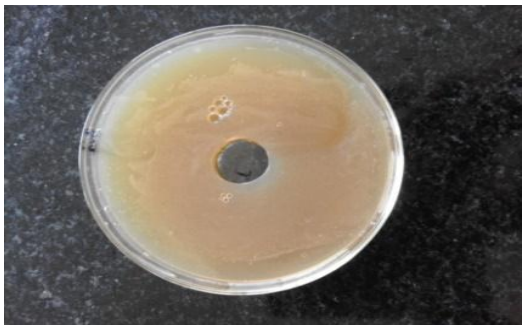
Control





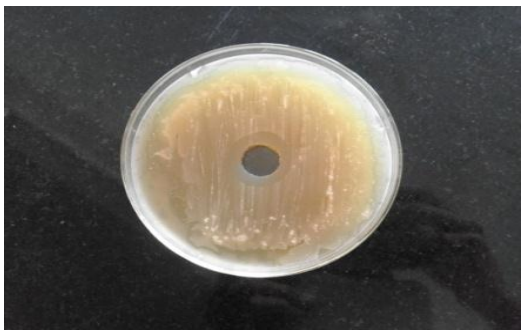
15 Min

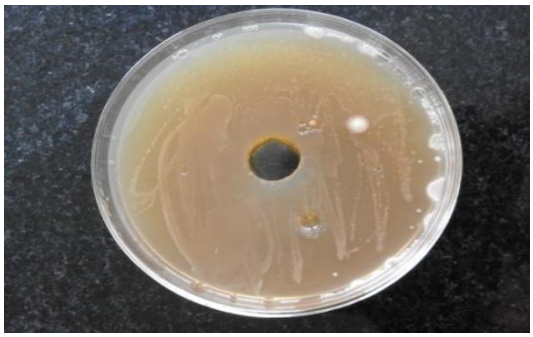




Sr.no.	Concentration of extract	Average zone of inhibition in mm
1.	20µg/ml	19mm
2.	100µg\ml	23mm

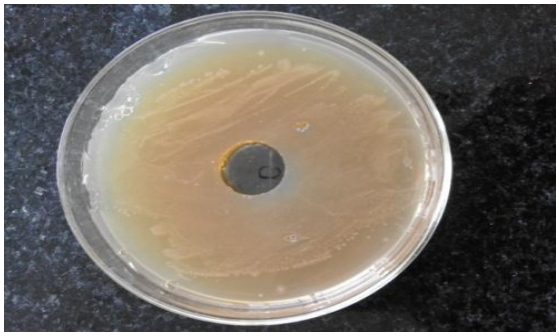
30 Min

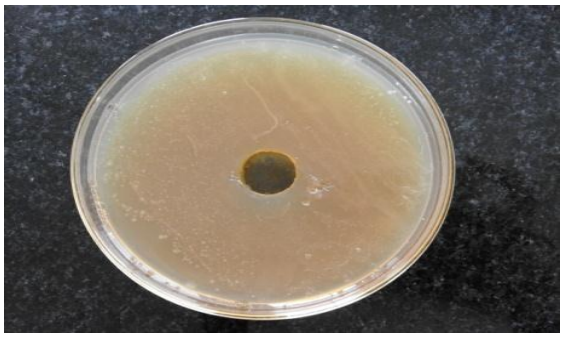




Sr.no.	Concentration of extract	Average zone of inhibition in mm
1.	20µg/ml	23mm
2.	100µg\ml	29mm

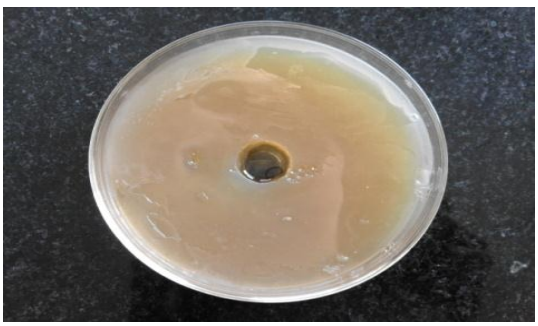
45 Min

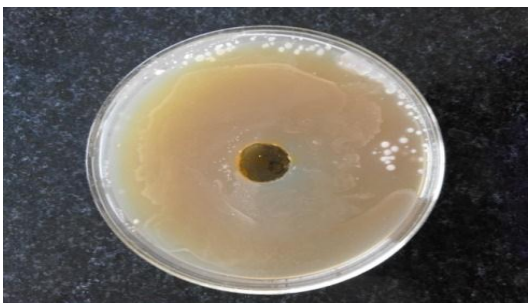




Sr.no.	Concentration of extract	Average zone of inhibition in mm
1.	20µg/ml	24mm
2.	100µg\ml	28mm

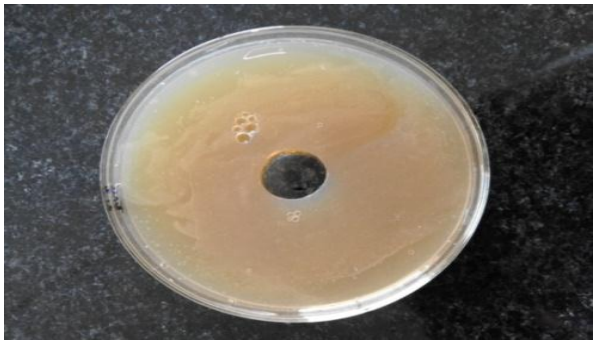
E. coli
Standard

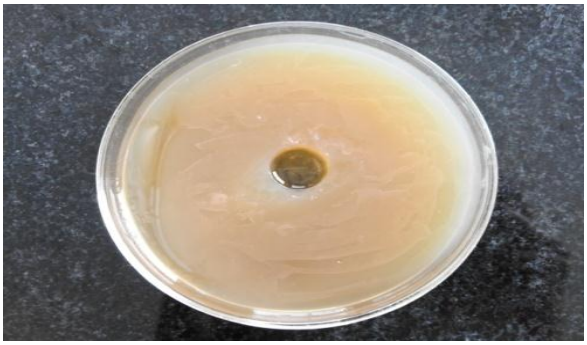




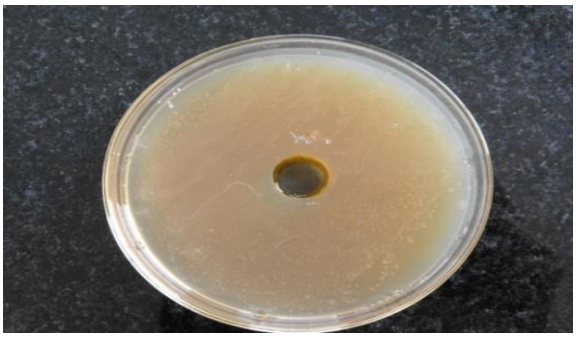
Sr.no.	Concentration of extract	Average zone of inhibition in mm
1.	20µg/ml	29mm
2.	100µg/ml	30mm

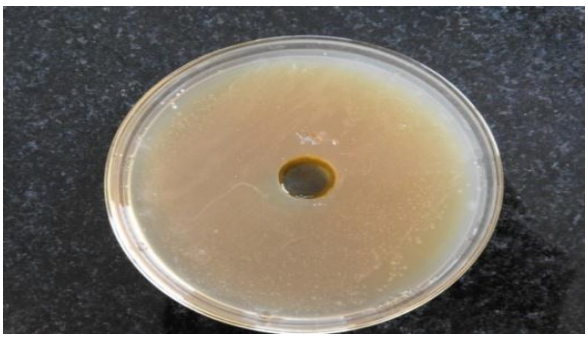
Control





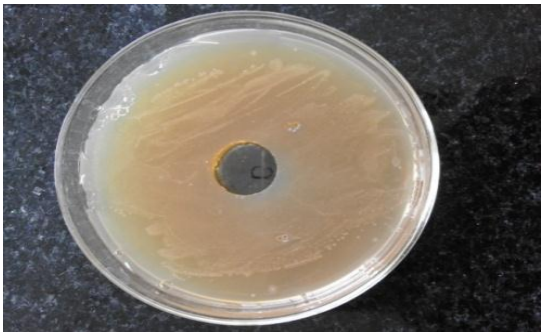
15 Min

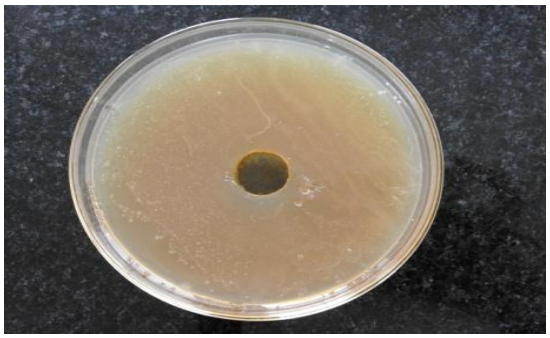




Sr.no.	Concentration of extract	Average zone of inhibition in mm
1.	20µg/ml	21mm
2.	100µg\ml	26mm

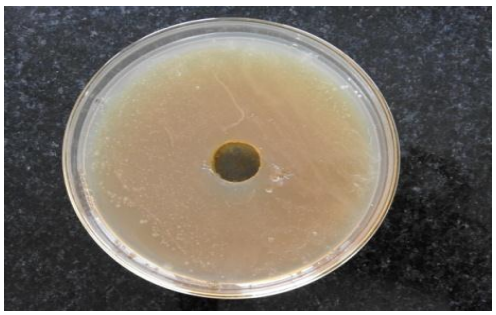
30 Min

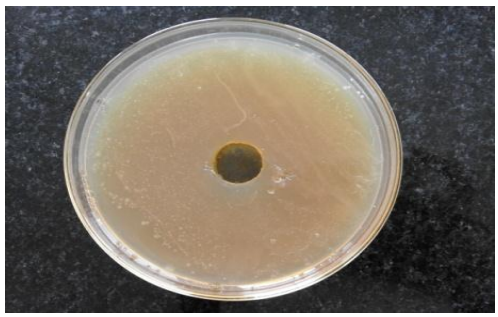




Sr.no.	Concentration of extract	Average zone of inhibition in mm
1.	20µg/ml	24mm
2.	100µg\ml	28mm

45 Min





Sr.no.	Concentration of extract	Average zone of inhibition in mm
1.	20µg/ml	26mm
2.	100µg\ml	31mm

RESULT

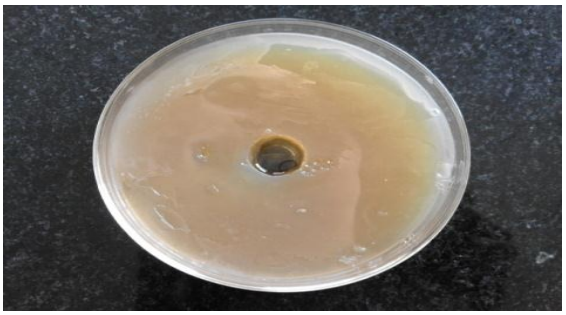
Sr No.	Time	% Practical Yield (w/w)
1	After 15 min	22.4%
2	After 30 min	41.6%
3	After 45 min	31.2%

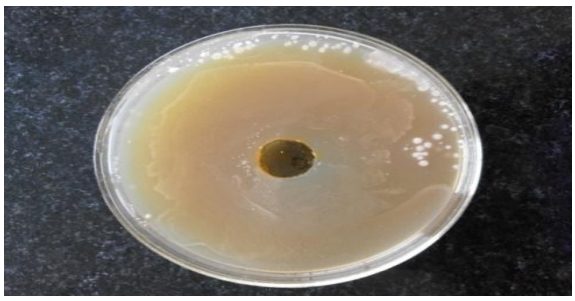
1. Percentage Yield extract
2. By performing phytochemical screening tests of extracts obtained after 15 min, 30 min and 45 min.extraction process. It was found found that *Mitragynaparvifolia (Roxb)korth* contains Alkaloids,Tannins,steroids,flavanoids and saponin glycosides.
3. Results of antibacterial activity:

Methanolic extract of *Mitragynaparvifolia (Roxb)korth*isprepared by ultrasonication method was found to be showed excellent antibacterial activity against E. Coli. Therefore, it can be concluded that ultrasonication method is also superior method for preparation of extract.

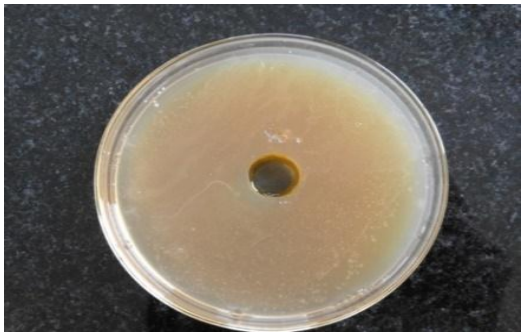
Sr No.	Time of extraction (min)	Average diameter of zone of inhibition (mm)	
		Minimum conc.	Maximum conc.
1.	Standard	29 mm	30 mm
2.	15 min	21 mm	26mm
3.	30 min	24 mm	28 mm
4.	45 min	26 mm	31 mm

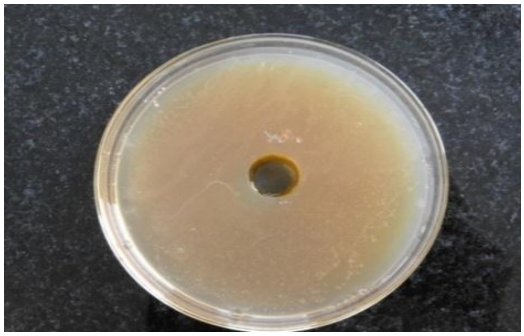
Zone of inhibition



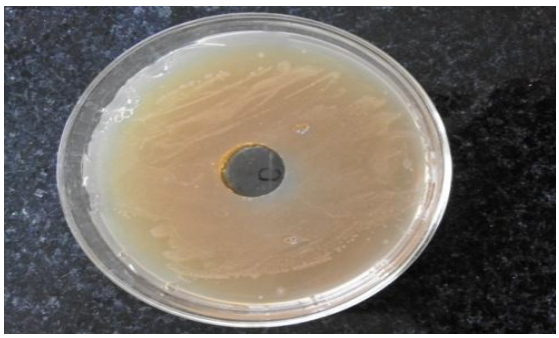


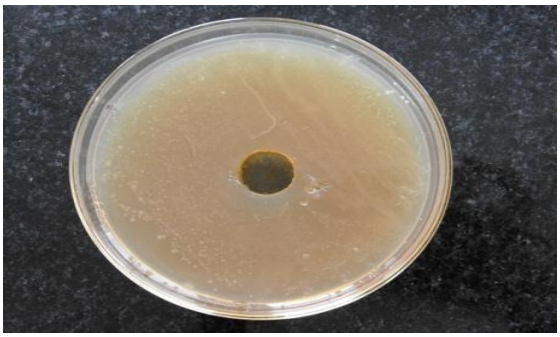
Zone of inhibition of standard drug



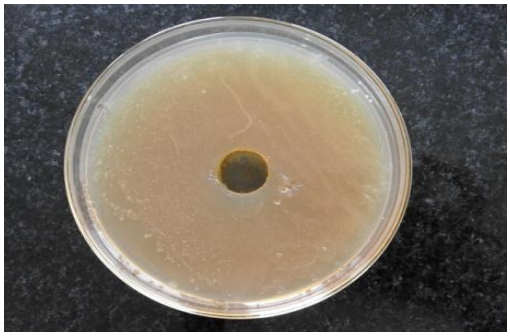


Zone of inhibition of after 15 min extract





Zone of inhibition of after 30 min extract



Zone of inhibition of after 45 min extract

CONCLUSION

Therefore, *MitragyanaParvifolia*(Roxb) korth is considered as a plant of various health benefit, the plant gives the good antibacterial activity with very less toxic side effect and new method of extraction (Ultra-sonication)is very easy and it gives best practical yield. The extract also showed antibacterial activities against *E.coli.* As the time of extraction increases the antibacterial activity also increases linearity.

REFERENCES

1. Gajendra Pratap Choudhary *et.al* A Review on*Mitragynaparvifolia (Roxb) korth*.An Indian Medicinal Plant. International Journal Of Pharmacy And Pharmaceutical Research, 2016; 7(1): 2349-7203.
2. Pundir Ram Kumar *et.al* Antimicrobial Activity of *Mitragynaparvifolia (Rox.)korth.* Barks and *ButeaMonosperma* Leaves Extracts Against Human Pathogenic Microbial Strains. International Journal Of Drug Development And Research, 2011; 3(4): 0975-9344.
3. Kaushik D. *et.al* Study of Analgesic and Antimicrobial Potential of *Mitragynaparvifolia (Roxb) korth.* International Journal Of Pharmaceutical Science And Drug Research, 2009; 1(1): 6-8.

4. PasupatVasmatkar *et.al* Antibacterial Activity and GC-MS-Analysis of Methanolic Extract from Stem Bark and Leaves of *Mitragynaparvifolia (Roxb)korth*.Indo American Journal of Pharmaceutical Reasearch, 2014; 2231-6876.
5. Supriya N. Mandrupkar*et. Al* Antihelmintic activity of *Mitragynaparvifolia (Roxb) korth*. fruit extract. World Journal of Pharmacy and Pharmaceutical Sciences., 2017; 2278-4357.
6. Khandelwal KR. *et. al*. Practical Pharmacognosy 22nd Edition, Nirali Prakashan., 2012.
7. Nikhil S. Dhane. et. Al Antibacterial Acivity of Methanolic Extract of Nycthanthesarbortristis. International Journal Of Pharmacology And Pharmaceutical Sciences., 2016; 3(4): 76-79.
8. Indalkar A.S.et.al Standardization and comparative phytochemical screening of *CucurbitaMaximas*different extracts obtained by different extraction techniques. World Journal of pharmacy and pharmaceutical sciences., 2017; 6(10): 694-701.
9. Bhokare Pallavi V. et. al comparative phytochemical screening of different extraction techniques and formulation, characterization of herbal lipsticks containing *Beta vulgaris Linn*. World Journal Pharmaceutical Research. 2017; 6(6); 751-764.