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Evaluation of Preliminary Phytochemical, Antimicrobial and Anthelmintic activity of Zizyphus oenoplia (L) Mill

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ABSTRACT

Only fewer medicinal plant shows Antimicrobial and Anthelmintic property in which Zizyphus oenoplia is one of them. The aim of present study was to investigate the preliminary phytochemical constituent and Antimicrobial and Anthelmintic activity of Zizyphus oenoplia against Microbes (bacterial suspention) and earthworm (Pheretima postuma). The concentrations (100, 200, 300 & 400 mg/mL) of each extracts were studied in activity, which involved the determination of Minimum Inhibitory Concentration by bacteria ,time of paralysis and time of death of the worms. Antimicrobial activity done by Disk diffusion Method and Broth Dilution Method . The total alcoholic extract and its ethanolic and aqueous fraction exhibited anthelmintic activity at highest concentration of 400 mg/mL. Albendazole in same concentration as that of extract was included as standard reference and normal saline water as control. So it could be confirmed that the total alcoholic extract, ethanol fraction and aqueous fraction of root of Zizyphus oenoplia showed significant anthelmintic properties comparable with standard drug, which is effective against parasitic infection of humans.

Key words: Zizyphus oenoplia, MIC, Antimicrobial, Anthelmintic property

INTRODUCTION

Many drugs commonly used today are of herbal origin. Indeed, about 25 percent of the prescription drugs dispensed in the United States contain at least one active ingredient derived from plant material. Some are made from plant extracts; others are synthesized to mimic a natural plant compound. The US herbal medicine consumption alone was worth US$ 17 billion in the year 2000 and the global market for herbal medicines today is estimated to be a whopping US$ 60 billion. The World Health Organization (WHO) estimates that 4 billion people, 80 percent of the world population, presently use herbal medicine for some aspect of primary health care. Major pharmaceutical companies are currently conducting extensive research on plant materials gathered from the rain forests and other places for their potential medicinal value.

Information about *Ziziphus Oenoplia Mill.*:

Ziziphus oenoplia (L.) Mill. is a spreading, climbing, thorny shrub. Ziziphus is a genus of about 40 species of spiny shrubs and small trees. The leaves are simple alternate, ovatelanceolate, asymmetric, denticulate, acute, oblique at base, entire, silky pubescent, with three prominent basal veins. The fruit is a globose drupe (fleshy VERNACULAR NAMES English Ziziphusoenoplia (L.) Mill. Hindi Makkay, Kokalber Telugu Parigi Sanskrit Karkandhu Tamil Curia Bengali Siyakul Kannada Barige, Challe Konkani Burgi Malayalam Vanthutali Marathi Burgi, Chinibor, Maastodi Oriya Kontaikoli Nepalese Aulebayar, Boksibayar P.Venkanna et al INTERNATIONAL JOURNAL OF ADVANCED PHARMACEUTICAL SCIENCES WWW.ijaps.net 27 exo and mesocarp with a hard endocarp), drups very pleasant to eat, black and shiny when ripe, containing a single seed. It ranges from the Indian subcontinent through southern China and South east Asia to northern Australia . It grows along roadside, forests and thickets.

Fig: Fruits and Leaves of *Ziziphusoenoplia mill.*

Kingdom-Plantae.

Order-Rosales.

Family-Rhamnaceae.

Genus-Ziziphus.

Species-Z.oenoplia.

Synonym: Makai in Hindi, Jackal Jujube, Small Fruited Jujube, Wild Jujube in English, Borkati in Marathi, Bahukantaka, Karkandhauh in Sanskrit, Shiyakul, Chiyakul, Banbaroi in Bengali, Pargi in Kannada. Kanher, Kantaikoli, Kul.

Family:-Rhamnaceae.

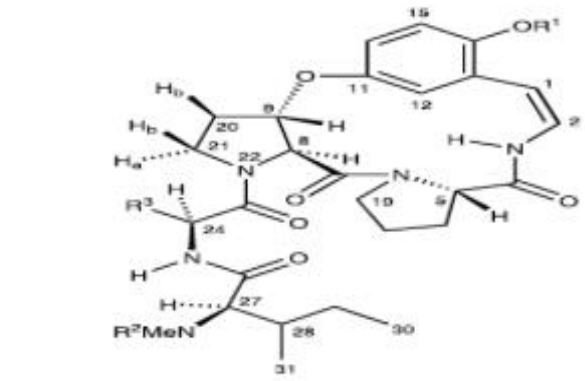
Macroscopy

It is an erect, thornystrggling or climbing shrub. The leaves are simple, etiolate and alternate or opposite, innately veined entire to serrate.The flowers are green,in sub sessile axillary cymes.The fruits are containing a single seed having globosedrupe, black and shiny when ripe. frruiting pedicel 3-4mm long, seeds 1cm long. Seeds are woody or horny. Rootsare cylindrical and brown in colour. Root possess root hairs. Inflorescence axillary shortly pedunculatecymes. Pedicels about 2mm long. Calyx lobes 1.5-2mm long, ovatetriangular, apex

acute, glabrous inside, brownish, apparently hairy outside. Petals 0.8-1mm long, spatulate, clawed, shorter than calyx. The flower and fruiting of the plant occurs in the month of August-January. It can be propagated by seeds.

Chemical Constituents-

Bark contains two new cyclopeptide alkaloid, Ziziphine-A, Ziziphine-B, etulinicacid,d-glucose, d-fructose, sucrose, unidentified polysaccharide. Stem bark contains Ziziphine(A-G), abyssinine-A,abyssinine-B, Ziziphine-A, Ziziphine-B, Ziziphine-C, Ziziphine-D, Ziziphine-E,Ziziphine-F,Ziziphne-G, Ziziphus-H.Leaves containsbetulinic acid, pentacyclictriterpene, methylated flavonol 3'4'-diOMequercetin.Root bark contains Alkaloid, 5-Carboxylic acid. Fruit contains Carbohydrate, Total Sugar, Moisture, Protein, Reducing Sugar, Non-Reducing Sugar, Carotenoid,Acid content, Ascorbic acid, Mineral.The roots of Ziziphus oenoplia plant contains Carbohydrate, Protein, Steroid, Alkaloid, Flavonoid, Amino acid, Tannins.



Ziziphine N: $R^1 = R^2 = Me$, $R^3 =$

Uses:

Fruits have medicinal properties like as blood purifier, abdominal pain killer, febrifuge. *Z.oenoplia* widely used in Ayurveda for the treatment of various diseases such as ulcer, stomach ache, obesity ,asthma and astringent, digestive, antiseptic, hepatoprotective, wound healing and diuretic property. *Ziziphusoenoplia* plant traditionally used as medicine for the treatment of various diseases such as digestive disorder,urinary troubles, diabetes, skin infections, diarrhoea, fever, bronchitis, liver complaints, anaemia. Roots used as astringent, bitter, anthelmintic, digestive, antiseptic. They useful in hyperacidity, ascaris infection, stomachalgia, healing of wound.Used as pacifies vitiated pitta, kapha, worms, pepticulcer,s tomachpain, sorethroat, dysentery, inflammation of uterus. Root and bark used to treat hyperacidity, stomachache. Leaves chewed and applied on wound. Nature is a

source of a medicinal agent since ancient times.Human life cannot be managed without herbal remedies. It is very clear that herbs are inexhaustible source of active ingredients [1].As plant derived medicines which have made large contributions to human health and well being although it is use for replacement of potent drug which is synthetic one [2]. Medicinal plants are isolated to obtain a chemical constituent or active ingredient[2]. Many studies are carried out to extract various natural products for screening antimicrobial activity and anthelmintic activity. The *ziziphusoenopliamill* are used to control severe diseases derived from bacteria and worms.

Gums and Mucilage

Gums are considered to be pathological products formed followinng injury to the plant or owing to unfavourableconditions, such as drought, by breakdown of cell walls (extra cellular formation; gummosis).Mucilage are generally normal products of metabolism, formed within the cells (intracellular formation) and/ or are produced without injury to the plants. Gum and Mucilage from diferent sources are easily collected in different seasons in large qantities due to simple production process involved. There is less chance of side and adverse effect with natural materials compared with synthetic one. e.g.povidone. Most Gums and Mucilages are obtained from edible sources. Normally, when gums and mucilage come into contact with water there is an increase in viscosity of formulation. Due to complex nature, it has been found that after storage there is reduced in viscosity. Gums are readily dissolves in water, whereas mucilage form slimy masses. Gums are pathological products and mucilage are physiological products. generally mucilage obtained from hydrocolloid plant. Linear polysacchride occupy more space and more viscous than highly branched compounds form gels more easily and more stable because extensive interaction along chains not possible.

RATIONALE:

Medicinal plants have different medicinal properties.They are resources of the drugs of traditional system of medicines, modern medicines, Neutraceuticals, food supplements etc. plants are very useful, self generating machines, producing a variety of useful bioactive products. The major part of the traditional therapy involves the use of plant extract and their active constituent.

Human infections, particularly those involving microorganisms i.e. bacteria,fungus,viruses;they cause serious infections in tropical and subtropical countries of the world.In recent years, multiple drug resistance in human pathogenic microorganism has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of such diseases.Plants are the richest source of the natural antimicrobial agents.Traditional healers claim that some medicinal plants are more efficient to treat infectious diseases than synthetic antibiotics. Biomolecules of plant origin appear to be one of the alternatives for the control of these antibiotic resistant human pathogens.

Different extracts from traditional medicinal plants have been tested.Many reports have showthe effectiveness of traditional herbs against microorganism, as a result, plants are one of the bedrocks for modern medicine to attain new principles.

Keeping this view; In current study medicinal herbs extract have selected for comparison of in-vitro antimicrobial activity against human pathogenic Escherichia coli and staphylococcus aureus by employing disc diffusion and broth dilution method and anthelmintic activity against worms(earthworm) by employing simple inoculation petri plate method.

2. Materials and methods

2.1.Chemicals

Ethanol, Distilled Water, Molisch's Reagent, Conc. Sulphuric acid, Ruthenium Red Solution, 0.2N Iodine Solution, Fehling's-A Solution, Fehling's-B Solution, 4% Sodium Hydroxide, 1% Copper Sulphate Solution, Chloroform, Wagner's Reagent.

2.2.Equipments

Analytical Balance, Hot Air Oven, Digital Microscopy, Sonicator, VacumFilteration assembly, Microwave Oven, Refrigerature, Incubator, Mechanical Blender, petri plates, stirrer.

2.3.Collection and Authentication

The fruits of *Ziziphusoenoplia mill* plants were used for the isolation of mucilage. These material were collected from the local area of vaduj, Maharashtra and Authenticated by Botanist from Y.C. Institute of Science, Satara. then fruits was collected and dried at sunlight for one week. collected fruits were powdered for 5 min in mechanical blender and pass through 40 no. seive. then powder of fruits stored at well closed container.

2.4.Method:

Isolation of Mucilage by Microwave Procedure:

Ziziphus oenoplia mill Fruits (100g) were powdered in a mechanical blender for 5min.and soaked in distilled water (500ml) for 24hr in 1000 ml beaker. It was kept in a microwave oven along with a glass tube inside to prevent bumping. It was subjected to microwave irradiation at 420W intensity for 7 min. The beaker was removed from the oven and kept aside for 2 hours for the release of mucilage into water. Equal volume of Ethanol was added to the filterate to precipitate the mucilage and kept inside a refrigerator for one day for effective settling. It was filtered and dried completely in an incubator, powdered and weighed.

Yield of the mucilage obtained from microwave method were calculated.

2.5.Organoleptic properties:

2.6. Phytochemical screening (Qualitative tests):

Prepration test solution :

Take 100mg of drug and dissolve it in 10 ml of distilled water and used for further confirmatory test.

Preliminary tests were performed to confirm the nature of mucilage obtained. The chemical tests such as: Ruthenium red test, Molisch'stest, Iodine test, Fehling's test, Biuret test, Wagner's test, Shinoda test.

2.7.Biological activity:

A] Antimicrobial screening:

2.7.1.MediaPrepration:

Sr.No.	Name of the Ingredients	Quantity given
1	Macconkey agar	12.6 gm
2	Nutrient agar	6 gm
3	Chloramphenicol	100 mg
4	Distilled water	500 ml
5	Methanol	20 ml

2.7.2: Collection of bacterial culture:

Bacteria used in this evaluation are *Staphylococcus aureus* and *Escherichia coli* collected from culture collection center.

2.7.3: Laboratory Equipments:

Incubator, Hot air oven, Refrigerator, Autoclave, Electrical water bath,

2.7.4: Apparatus:

Petri plates, porciline dishes, nichrome wire loop, conicalflask, beakers,

2.7.5: Inoculum Preparation:

Bacterial inoculum was prepared by inoculating a loopful of test organisms in 5 ml of Nutrient broth and incubated at 37°C for 3-5 hours till a moderate turbidity was developed.

2.7.6:Preparation of concentration for antimicrobial assay:

Weigh about 100 mg of mucilage of *ziziphusoenoplia mill*. Dissolve it into 100 ml of distilled water to make a concentration 1000ug/ml.

2.7.7: Antimicrobial activity susceptibility test:

Disk diffusion test and Agar dilution test are adopted for the evaluation of antimicrobial activity

a] Disk diffusion Method:

Antibacterial activity of the different extracts was determined by disk diffusion method on nutrient agar medium (Anon, 1996). Wells are made in nutrient agar plate using cork borer (5 mm diameter) and Inoculums containing 106 CFU/ml of bacteria were spread on the solid plates with a sterile swab moistened with the bacterial suspension and fifty micro-litres of the working suspension/solutionof different medicinal plant extract and same volume of extraction solvent for control was filled in the wells with the help of micropipette. Plates were left for some time till the extract diffuse inthe medium with the lid closed and incubated at 37°C for 24 h. After over night incubation the plates were observed for the zone of inhibition (ZI) and the diameter of the inhibition zone were measured using scale and mean were recorded [7].

b] Broth Dilution Method:

The antimicrobial to be tested is added to broth, which is then placed into dilution plates and diluted with varying levels of water. After this,the pathogen to be tested is added to each plate,plus a control plate that does not receive any antimicrobial. The dilution plates are then incubated at a temperature of 37 degrees Celsius. The plates are then incubated for 16 to 18 hours, although incubation time may be less for bacteria populations that divide quickly. After incubation, the plates are examined to determine if bacterial expansion has occurs. The lowest concentration of antimicrobial that stopped the spread of the bacteria is considered to be the minimum inhibitory concentration of those bacteria[28]

2.7.8.Determination of Minimum Inhibitory Concentration:

The minimum inhibitory concentration (MIC) of the fruits mucilage of ziziphusoenoplia mill were determined by micro broth dilution method. For MIC two-fold serial dilution of the extract were prepared (100, 200 and 300ug/ml) in wells. Incubation of plates was carried out at 37 degrees Celsius for 18-24 hours. The minimum inhibitory concentration for ziziphusoenoplia mill were found to be 100 ug/ml, 200 ug/ml and 300ug/ml respectively.

B] Anthelmintic activity:

2.7.1.Worm collection:

Anthelmintic activity performed on adult indian earthworm pheretimaposthuma as it has anatomical and physiological resemblance with intestinal round worm. Indian earth worm collected from moist soil and cleaned with tap water to remove all dirt matter.

2.7.2:Preparation of concentration for anthelmintic assay:

Weigh about 100mg, 200mg, 300mg, 400mg of mucilage of *ziziphus oenoplia mill*. Dissolve it into 50 ml of distilled water to make a concentration.

2.7.3. PROCEDURE:

Indian earth worm was collected from moist soil and washed with normal saline. The earth worm of 6-8 cm was used for experimental protocol. The worms were divided into five group containing six earthworms in each group. Six equal size worms were released in each 50 ml formulation containing different concentration of mucilage The solution of mucilage concentration 100mg ,200mg, 300mg, 400mg was prepared. Groups of approximately equal size worms consisting of six earthworm individually in each group were released into in each of desired concentration of mucilage in the petridish. The anthelmintic activity was performed according to standard screening methods. oneindian earth worms (adult) positioned in petridish containing 15 ml contained 20, 40, 60 mg/ml plant extracts in three different petridish. Every petridish was placed with 5 earth worms and studied for paralysis or death. The mean time for paralysis was recorded when no movement of any sort could be observed, the time to death of worm (min) was recorded after ascertaining that worms not moved even with external physical stimuli. The test results were compared with reference compound Albendazole 10, 20, 30, 40 mg/ml tested samples. The procedure was repeated 3 times verify the reading.

Observation:

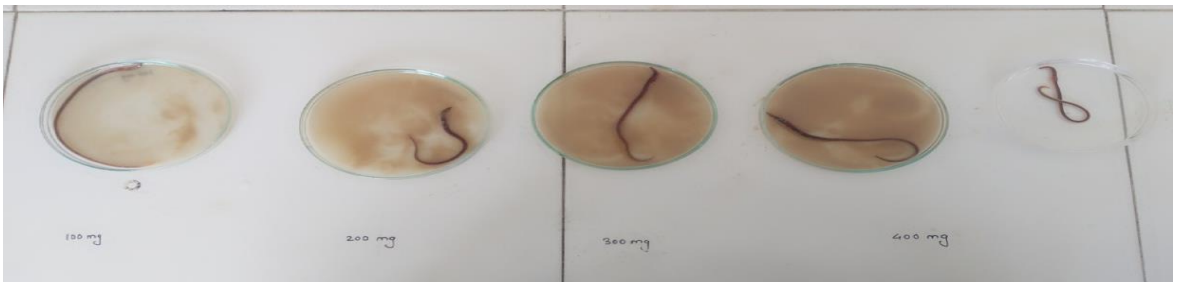


Fig: in-vitro study of anthelmintic activity.

3.Results:

3.1.Table no.1 Quantity of mucilage obtained after isolation procedure

Sr.No.	gm of powder taken for isolation procedure	Quantity of mucilage in gm
1	100gm	12gm

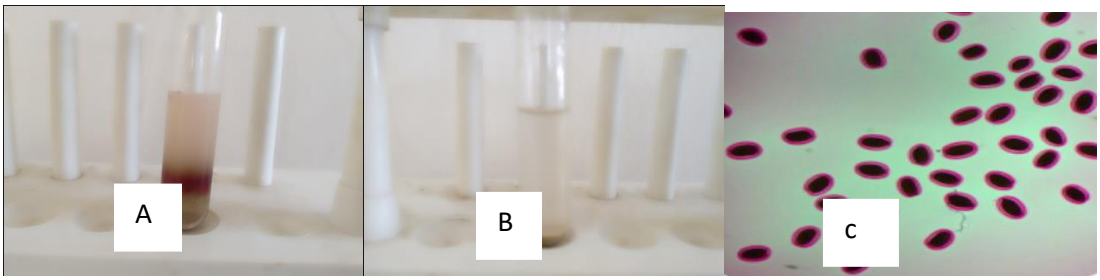
3.2Table no.2 physicochemical and organoleptic properties:

Colour	Odour	Taste	Fracture	Texture
Brown	Odourless	Characteristic	Rough	Irregular

3.3.Table no.3 preliminary and confirmatory test for mucilage:

Sr. No.	Test	Observation	Inference
1.	Test Observation Inferences Molisch's test: (100 mg dried mucilage powder + Molisch's reagent + conc. H2SO4 on the side of a test tube)	Violet green color observed at the junction of the two layers.	Carbohydrate present
2.	Ruthenium test: Take a small quantity of dried mucilage powder, mount it on a slide with ruthenium red solution, and observe under microscope.	Pink colour develop.	Mucilage present
3.	Iodine test: 100mg dried mucilage powder + 1 ml 0.2 N iodine solution.	No colour observed in solution.	Polysaccharides present (starch is present)
4.	Mix.1 ml of fehlings A and fehlings B solution boil for 1 min add equal volume of test solution heat inboiling water bah for 5 to 12 min.	First yellow then brick red ppt.	Redusing sugar absent.
5.	Biuret test-To 3ml test solution add 4% NaOH and few drops of 1% CuSO4 solution	Violet or pink colour.	Protein absent.
6.	SSalkawaski test-2 ml of extraatadd 2 ml of chloroform and 2 ml of conc. H2SO4 and shake well.	Chloroform layer red acid layer and greenish yellow fluorescence.	Steroid absent.
7.	Test for alkaloids- Prepration of test solution-evaporate aq. Alcoholic extract add dil.HCL shake well and filter. Wagners test-2-3 ml filtrate with few drops of wagners reagent.	Reddish brown ppt.	Alkaloid are absent
8.	Shinoda test-To dry powder or extract add 5 ml 95% ethanol,few drops of conc.Hcl and 5g og magnesium turnings.	Orange pink or purple colour.	Flavanoids are absent.

Observations:



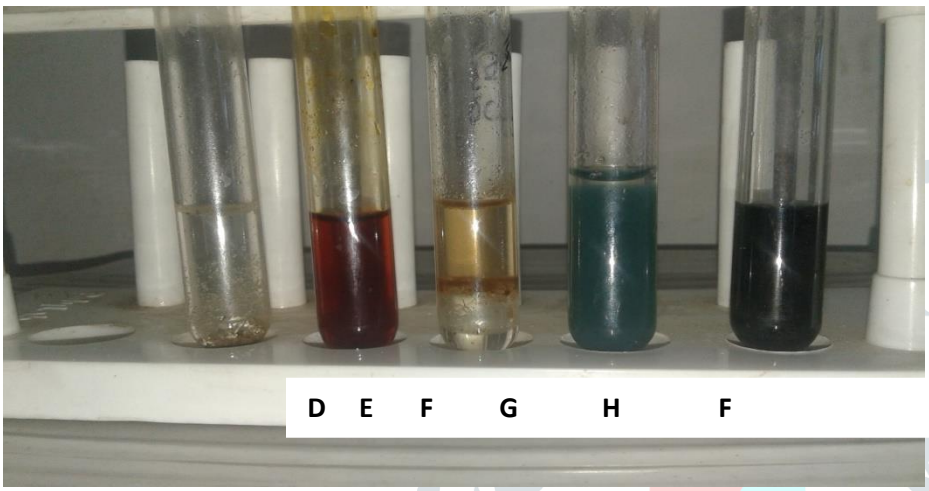


Image: Qualitative test.

A]Molisch test, B]Iodine test, C]Ruthenium red test, D]Fehlings test, E]Biuret test, F]Salkawaski test, G]Alkaloid tests, H]Shinoda test.

3.4.tableNo 4. Antimicrobial activity of mucilage against microbial pathogen by disk diffusion method.

Mucilage Conc./ml.	**Diameter of Zone of inhibition (mm)**
100ug/ml	19
200ug/ml	24
Standard(chloramphenicol) 100ug/ml	27

Observations:

Fig.zone of inhibition by disk diffusion method

Antibacterial activity of prepared mucilage of *ziziphusoenoplia mill* were studied by antimicrobial susceptibility tests disk diffusion method. Results of the disk diffusion study and zone of inhibition shown inabove diagrams.

Table no.3.5: Antimicrobial activity of prepared mucilage against microbial pathogen by broth dilution method.

Concentrations (ug/ml).	**Diameter of zone of inhibition in mm**
100 ug/ml	7
200 ug/ml	11
Standard100 ug/ml	13

Observations:

Antibacterial activity of prepared mucilage of *ziziphusoenopliamil*l were studied by antimicrobial susceptibility tests broth dilution method. Results of the broth dilution study and zone of inhibition shown in above diagrams

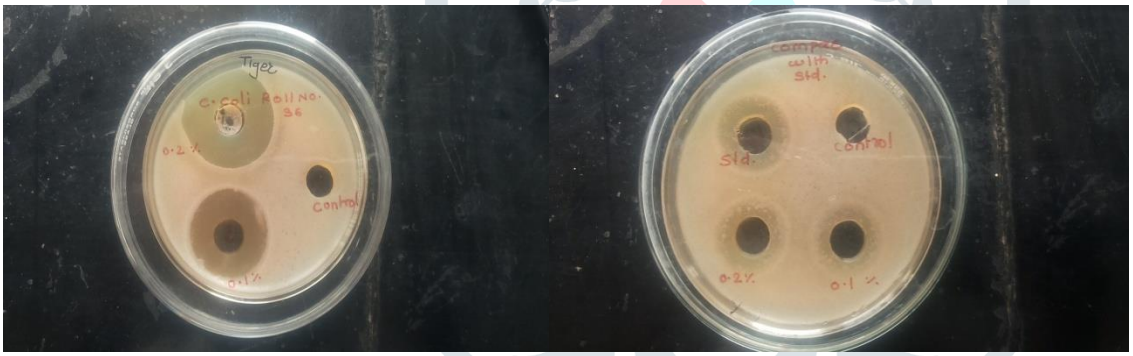


Fig.1 zone of inhibition without comparing with standard.
Fig.2 zone of inhibition compairing with standard.

Sr. no.	Earthworm set no.	Time taken for paralise (hours.minute.second)					Time take for death (hours.minute.second)				
	Conc. Of mucilage.	100mg	200mg	300mg	400mg	Albendazole (std.)	100mg	200mg	300mg	400mg	Albendazole (std)
1	1	3.38.27	3.11.09	2.48.11	2.12.18	2.14.26	3.52.53	3.27.06	2.59.08	2.27.21	2.30.33
2	2	3.49.11	3.31.47	3.03.18	2.35.27	2.37.13	3.59.09	3.41.59	3.21.29	2.48.33	2.51.23
3	3	3.41.06	3.29.43	3.02.34	2.23.45	2.25.43	3.52.54	3.36.23	3.19.03	2.32.34	2.35.45
4	4	3.38.27	3.11.01 9	2.48.11	2.12.14	2.14.11	3.51.53	3.27.06	2.59.08	2.27.21	2.31.12
5	5	3.39.11	3.21.47	3.03.18	2.25.38	2.30.35	3.49.09	3.31.59	3.21.29	2.38.33	2.42.24
6	6	3.31.06	3.29.53	3.12.34	2.21.11	2.23.23	3.42.54	3.36.23	3.24.03	2.22.34	2.26.34
	Mean	3.45.29	3.26.36	2.39..26	2.27.22	2.29.23	3.50.44	3.33.21	3.03.28	2.32.33	2.37.56
	Standard deviation(S)	±4	±6	±7	±3	±3.5	±4	±6	±8	±7	±6.2

4: Counclusion:

The literature supports presence of primary metabolites carbohydrates, proteins , fats and polysaccharides in larger amount and secondary metabolite like alkaloids, flavanoids, steroids and terpenoidsin smaller amount in *Ziziphusoenoplia Mill.*[7,8]. Mucilage was isolated from fruits of *Ziziphus oenoplia* collected from the local area tahasil vaduj, Maharashtra (India) and authenticated by Botanist from Y.C.Institute of Science, Satara. Presence of polysaccharides was confirmed from microscopical test with Rhuthenium red solution.[13]The more energy efficient microwave assisted extraction of mucilage given better yield 12 % w/w. The various concentrations of aqueous solution of mucilage of Z.*oenopliaMill*showed excellent antibacterial activity in comparison to the standard chloramphenicol against *Staphylococcus aureus* and *Escherichia coli*. The *Z.oenoplia*also showed anthelmintic activity against Indian earthworm. The plant significantly possesses anthelmintic activity, at concentration 40mg/ml and showed time required for death as 2.32.33 hours. minute. Seconds in comparison to standard albendazole 2.37.56 at 40 mg/ml concentration. It was concluded finally that effective and safe herbal

antibiotic and anthelmintic may be produced in the future from fruit mucilage of *Z. oenoplia*. There is future scope for screening the isolated mucilage for other pharmacological activities.

5.Acknowledgments:

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6.TRANSPARENCY DECLARATION:

The authors declare that there is no conflict of interest regarding the publication of this article.

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