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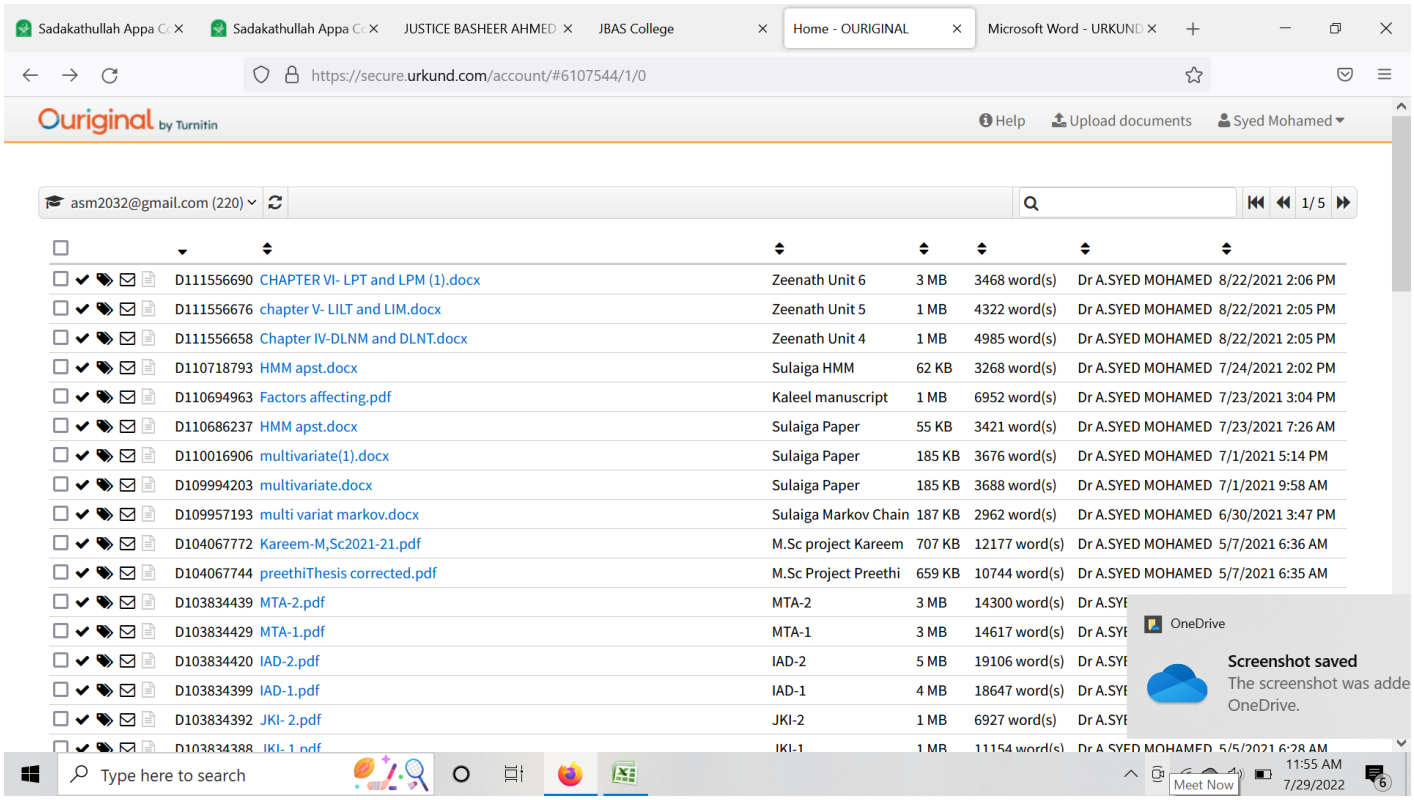
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ANTIMICROBIAL ACTIVITIES USING CISSUS QUADRANGULARIS PLANT STEM EXTRACT A Project work submitted to Sadakathullah Appa College (Autonomous), (Re-accredited with "A" Grade by
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DECLARATION I hereby declare that the thesis entitle, " Antimicrobial activities using Cissus quadrangularis plant stem extract" submitted by me for the Degree of Bachelor in Chemistry is the res
original and independent research work carried out under the guidance of Dr. A. SYED MOHAMED, Assistant Professor and Head, Research Department of Chemistry, Sadakathullah Appa Colleg
(Autonomous), Tirunelveli and it has not been submitted for the award of any degree, diploma or associate ship, fellowship of this or any other University or any other Institution. Place: Tirunelve
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quadrangularis 3.2 Some pharmacological activities of Cissus quadrangularis stem 3.3 Secondary Metabolites of Cissus quadrangularis 4. CHAPTER- 4 MATERIALS AND METHODS 4.1 Anti-bacte
Antimicrobial activity test (Disk diffusion method) 4.2.1 Taxonomial classification of Escherichia coli 4.2.2 Taxonomical classification of Bacillus subtilis
4.2.3 Taxonomical classification of Pseudomonas aeroginosa 4.2.4 Taxonomical classification of Staphylococcus 4.3 Preparation of plant extracts 4.4 Preliminary phytochemical analysis 4.5 Pack
gel chromatography column 5. CHAPTER-5 RESULTS AND DISCUSSION 5.1 Ethanol extract of Cissus quadrangularis 6. CHAPTER-6 CONCLUSION 7. CHAPTER-7 REFERENCES
Anti- Bacterial studies using Cissus quadrangularis Page 1 INTRODUCTION
Anti- Bacterial studies using Cissus quadrangularis Page 2 CHAPTER- I INTRODUCTION

95% **MATCHING BLOCK 2/17** W

INTRODUCTION Medicinal Plants have been used as a source of medicine since the dawn of civilization. The plant designed as medicinal is implied that it is useful as a drug or therapeutic agent ingredient of a medicinal preparation. Various medicinal plants have been applied for years in daily life to treat disease all over the world 1 . The stem contains two unsymmetrical tetracyclic trite two steroidal principles. The presence of β-sitosterol, δamyrin, δ-amyrone, and flavonoids (quercetin) having different potential metabolic and physiological effects have also been reported 2 . T protective effect of a methanolic extract of C. quadrangularis was similar to that of the reference medicine sucralfate 2 . Many imitate agents such as estrogens in hormone replacement therapy estrogen receptor modulators have been designed to treat osteoporosis but each one of them is linked with side effects such

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as hypercalcemia, hypercalciurea, raised risk of endometrial, and breast cancer, breast tenderness, menstruation, thromboembolic events, vaginal bleeding and hot flushes 3 .

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The use of plant extracts and phytochemicals, with known antibacterial properties, may be of immense importance in therapeutic treatments.

India is rich in various medicinal plants and also familiar for its traditional medicinal systems - Ayurveda, Siddha, and Unani. Ancient Indian medicinal system of health care
Anti- Bacterial studies using Cissus quadrangularis Page 3 focused on views of man and his illness. It has been pointed out that the healthy human means metabolically well-balanced human be offered programs to rejuvenate the body through diet and nutrition and also treatment methods to cure many common diseases such as food allergies, which have few modern treatments. In Ir 20,000 medicinal plants have been documented. The beginning of modern drug research in India can be traced to early part of the twentieth century. Colonel Ram Nath Chopra is recognized a pharmacology, pioneer of systematic studies of indigenous drugs, promoter of Indian systems of medicine, and patron of pharmacy. The lead given by Chopra led to start of investigation on indi plants in India. The twentieth century became the most important time in the history of chemistry, during this time synthetic chemistry dominated pharmaceutical industry where natural extract by the synthetic molecules that often had no connection to natural products. At twenty first century, exclusively of flowering plant origin 11% of the drugs were obtained which are considered as essential. Herbs played an important role in traditional medicine 4 . Alkaloids, flavonoids, tannins, and phenolic compounds are considered as the most important biologically active constituents recent years, attention has been directed in utilizing natural antioxidants substantially (Shahidi et al., 2006; Rajan et al., 2009). One such important medicinal plant
Anti- Bacterial studies using Cissus quadrangularis Page 4 is Cissus quadrangularis, the parts of these plants have lot of medicinal properties, which are traditionally used by people. Cissus quadra
Plant Morphology:

91% **MATCHING BLOCK 5/17** W

Kingdom: Plantae Clade: Tracheophytes Clade: Angiosperms Clade: Eudicots Clade: Rosids Order: Vitales Family: Vitaceae Genus: Cissus Species:

C. quadrangularis Fig. 1 Cissus quadrangularis
Anti- Bacterial studies using Cissus quadrangularis Page 5 Cissus quadrangularis belongs to the vitaceae family and is found in South East Asia.

83% **MATCHING BLOCK 6/17** W

It is a fleshy, cactus-like climber widely used as a common food item in India. This plant

has tri terpenoids, steroids 6,7 stilbenes 8 , flavanoids lipids and several catalpols 9 . The plant which has specific bone fracture healing properties which was approved by ancient Ayurvedic litera is also useful in the treatment of

100% **MATCHING BLOCK 7/17** W

helminthiasis, anorexia, dyspepsia, colic, flatulence, skin diseases, leprosy, hemorrhage, epilepsy, convulsion, haemoptysis, tumors, chronic ulcers, swellings.

Fig .2 Stem of Cissus quadrangularis
Anti- Bacterial studies using Cissus quadrangularis Page 6

90% **MATCHING BLOCK 9/17** W

The roots and stems are most useful for healing of fracture of the bones. The

stem is given internally and applied topically in broken bones, used in complaints of the back and spine. A paste of stem is useful for muscular pains. The stem juice of plant is used to treat scurvy disorders, otorrhoea and epistaxis. A paste of stem is given in asthma, burns and wounds, bites of poisonous insects and for saddle sores of horses and camels 10 . The stem boiled in limewater i preserve useful as a stomachic.
Anti- Bacterial studies using Cissus quadrangularis Page 7 AIMS AND OBJECTIVES
Anti- Bacterial studies using Cissus quadrangularis Page 8 CHAPTER- II AIMS AND OBJECTIVES 2.1 AIM: To prepare the extract of Cissus quadrangularis stem with using of ethanol. 2.2 OBJECTIV prepare crude extract from the stem of Cissus quadrangularis using solvent as ethanol. 2. To analyze the phytochemical component in the stem extract of Cissus quadrangularis. 3. To study the a activity of Cissus quadrangularis stem extract. 4. To separate the chemical compounds using column chromatography. The main purpose of this study was to investigate the effect of various cor including humidity, contact time, adsorbate concentration, and temperature on the adsorption of toluene vapor. Also, in the current study, the antimicrobial potential of ethanolic extract of Cissu quadrangularis roots was investigated.
Anti- Bacterial studies using Cissus quadrangularis Page 9 REVIEW OF LITRATURE
Anti- Bacterial studies using Cissus quadrangularis Page 10 CHAPTER - III REVIEW OF LITRATURE 3.1 Different names of Cissus quadrangularis: LANGUAGE NAMES Marathi Ghanasvel , Ghonuska Mhasvel Hindi Harajora Sanskrit Asthibhanga , Asthisamhara, Chaturdharin, Chitrakandali, Kandalata, Vajravalli Bengali Harajora Telugu Gudametige , Nalleruvajravalli Gujarati Asthisrnkhala (or) Ha பிரண்டை (அல்லது) வச்சிரவல்லி
Anti- Bacterial studies using Cissus quadrangularis Page 11 3.2 SOME PHARMACOLOGICAL ACTIVITIES OF CISSUS QUADRANGULARIS STEM Antioxidant and free radical scavenging activity: Ciss quadrangularis contains β- carotene, exhibits strong antioxidant and free radical scavenging activity can be explained with the help of methanol extract of the plant 11, 12 . Antibacterial activity: E obtained from Methanol extract (90%) and dichloromethane possess antibacterial activity against S. aureus, E. coli, and P. aeruginosa and mutagenicity against Salmonella microsome 13 . Bone h

65% **MATCHING BLOCK 8/17** W

Extract of the plant prepared by using alcohol, intramuscularly facilitates rapid healing of fracture in albino rats 14 95% Ethanol extract

of the plant possess antiosteoporotic activity. Anti-ulcer activity: Methanol extract produce of the plant shows healing effect on aspirin induced gastric mucosal damage through its antioxidative . Triterpenoids and β-sitosterol present in the plant methanol extract possess anti-lipid peroxidating effect and used to prevent gastric damage 16 . Analgesic, anti-inflammatory and stimulatory a Methanol extract of the plant contains flavonoids especially luteolin and by β-sitosterol, which are capable of showing the following activities such as analgesic, anti-inflammatory and venotonic associated with hemorrhoids, anti-inflammatory 17 .
Anti- Bacterial studies using Cissus quadrangularis Page 12 Toxicology: Cissus quadrangularis extract does not show any toxic effect on oral administration. The drug prepared by using this plant higher dose for a prolonged duration of treatment was confirmed by Toxicological evaluation of the plant. 3.3 Secondary Metabolites of Cissus quadrangularis Ethanol extract of Cissus quadrang screen all phytocomponents by using Gas Chromatography method revealed the presence of some of the medicinally valuable compounds like Asarone, Phytol, Phenol (Sathyaprabha et al, 201 extract Of the stems of Cissus quadrangularis showed three known compounds lupeol, freidalin and ?-sitosterol (Rao etal.,2011). Asarone Phytol

Anti- Bacterial studies using Cissus quadrangularis Page 13 Phenol Lupeol Freidalin freidalin upeol ?-sitosterol
Anti- Bacterial studies using Cissus quadrangularis Page 14 MATERIALS AND METHODS
Anti- Bacterial studies using Cissus quadrangularis Page 15 CHAPTER IV MATERIALS AND METHODS 4.1 Anti-bacterial activity The anti-microbial activity was analyzed by Disc Diffusion Method.
diffusion method is simple and reliable test to find out the effect of a particular substance on a specific bacterium. Microorganisms used: The antimicrobial activity was tested against the gram p
gram negative pathogens such as Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus. 4.2 Antimicrobial activity test (Disk diffusion method): The disk diffusion test, or ag
test, or Kirby–Bauer test (disc-diffusion antibiotic susceptibility test, disc-diffusion antibiotic sensitivity test, KB test), is a test of the antibiotic sensitivity of bacteria. It uses antibiotic discs to test th
which bacteria are affected by those antibiotics. In this test, wafers containing antibiotics are placed on an agar plate where bacteria have been placed, and the plate is left to incubate. If an antib
bacteria from growing or kills the
Anti- Bacterial studies using Cissus quadrangularis Page 16 bacteria, there will be an area around the wafer where the bacteria have not grown enough to be visible. This is called a zone of inhibi
Pathogens like Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus were prepared. 2. Nutrient agar plates were prepared and kept for drying. 3. Pathogens were taken in
4. Each swabs were incorporated separately in separate nutrient agar plate. 5. Sterile disk (6 mm) were placed in the agar plates. 6. 10 µl of samples were impregnated into the sterile disk, contro
mentioned using solvent. 7. Kept for 24 hours incubation at 37 °C. 8. After 24 hours the plates were taken and zone of inhibition were measured using ruler. Bacteria used: ? Escherichia coli ? Pse
aeroginosa ? Bacillus subtilis ? Staphylococcus
Anti- Bacterial studies using Cissus quadrangularis Page 17 4.2.1 Taxonomial classification of Escherichia coli Kingdom Bacteria Phylum Proteobacterea Class Gammaproteobacteria Order Entero
Family Enterobacteraceae Genus Escherichia Characteristic features of Escherichia coli: Escherichia coli (commonly abbreviated E.coli) is a Gram-negative, facultatively anaerobic, rod shaped ba
commonly found in the lower intestine of warm-blooded organisms (endotherms). Most E.coli strains are harmless, but some serotypes can cause serious food poisoning recalls due to food co
The harmless strains are part of the normal flora of the gut and can benefit their hosts by producing vitamin K2, and preventing colonization of the intestine with pathogenic bacteria. E.coli and
facultative constitute about 0.1% of gut flora, and feacal-oral transmission is the major route through which pathogenic strains of the bacterium cause disease. Cells are able to survive outside th
Anti- Bacterial studies using Cissus quadrangularis Page 18 a limited amount of time, which makes them ideal indicator organisms to test environmental samples for faecal contamination. There
growing body of research that has examined environmentally persistent E.coli which can survive for extended periods outside of the host. 4.2.2 Taxonomical classification of Bacillus subtilis King
Domain Bacteria Phylum Firmicutes Class Bacilli Order Bacillales Family Bacillaceae Genus Bacillus Species B. subtilis Characteristics features of Bacillus subtilis Bacillus subtilis cells are rod-shap
positive bacteria that are naturally found in soil and vegetation. Bacillus subtilis grow in the mesophilic temperature range. The optimal temperature is 25-35 ? Celsius (Entrez Genome Project). S
starvation are common in this environment, therefore, Bacillus subtilis has
Anti- Bacterial studies using Cissus quadrangularis Page 19 evolved a set of strategies that allow survival under these harsh conditions. One strategy, for example, is the formation of stress-resista
4.2.3 Taxonomical classification of Pseudomonas aeroginosa: Kingdom Bacteria Phylum Proteobacterea Class Gammaproteobacteria Order Pseudomonadales Family Pseudomonadaceae Genu
Pseudomonas Characteristics features of Pseudomonas aerogenosa Pseudomonas aeroginosa is a common Gram-negative rod-shaped bacterium that can cause disease in plants and animals,
humans. A species of considerable medical importance, P.aeroginosa is a prototypical "multidrug resistance (MRD) pathogen" that is recognized for its ubiquity, its intrinsically advanced antibiotic
mechanisms, and its association with serious illness, especially nosocomical infections such as ventilator, associated pneumonia and various sepsis sundromes.
Anti- Bacterial studies using Cissus quadrangularis Page 20 The organism is considered opportunistic insofar as serious infection is often superimposed upon acute or chronic morbidity, most n
fibrosis and traumatic burns – or fund in immuno-compromised individuals, but the organism does produce a range of clinically important infections in the immune- component and in situatio
pre-existing vulnerability is required e.g., hot tub folliculitis. 4.2.4 Taxonomical classification of staphylococcus: Kingdom Bacteria Phylum Firmicutes Class Bacilli Order Bacillales Family Staphylo
Genus Staphylococcus Characteristic feature of Staphylococcus: The genus is named by bacteriologist Alexander Ogston in 1882. It is derived from the Latin word meaning bunch of grapes in s
bacterium. They include at least 40 species. Two sub species are in nine species and three subspecies in one species and four subspecies in one species. In these, most of them are harmless. Th
Anti- Bacterial studies using Cissus quadrangularis Page 21 have a cell wall structure and G+C content of DNA in a range of 30-40 %. They grow in the presence of bile salts. The genus staphylo
colonizes the skin and upper respiratory tracts of mammals and birds. It can cause a wide variety of diseases in human and animals through either toxin production or penetration. They are com
food poisoning, they can be produced by bacteria growing in improperly stored food items. The most common sialadenitis is caused by staphylococci, as bacterial infections. Antibiotics is basic
are commonly cephalosporins, nafcillin, sulfadurgsvancomycin. Vancomycin is highly used.
Anti- Bacterial studies using Cissus quadrangularis Page 22 Fig 3. Laminar Air Flow Chamber used for Microorganism culturing Fig 4.Preparation of agar nutrient
Anti- Bacterial studies using Cissus quadrangularis Page 23 Fig 5. Prepared Nutrient Medium Fig 6.Solidified form of agar in petri dish
Anti- Bacterial studies using Cissus quadrangularis Page 24 Fig 7. Preparation for Disc Diffusion Method
Anti- Bacterial studies using Cissus quadrangularis Page 25 4.3 Preparation of plant extracts: Fresh stem samples Cissus quadrangularis were washed under running tap water and dried for 48 hr
oven at 60°C. Dried stem sample were grounded using an electric blender to obtain a fine powder and stored in polythene bags until needed for analysis. 30 g of the sample is soaked in the wat
solution was vigorously shaken at room temperature for 48 hrs. and was filtered with Whatmann No.1 filter paper. The filtrate is used for the phytochemical analysis. Chemical tests for screening
identification of bioactive chemical constituents in the Cissus quadrangularis species were performed in extracts using the standard procedures. 4.4 Preliminary phytochemical analysis : 5g of th
powdered stems of Cissus quadrangularis was successively extracted with petroleum ether (40-60 ? C), methanol, acetone and chloroform. All these extracts were subjected to preliminary phyt
analysis. The following tests were carried out. The results are presented in Table 3. a)Test for steroids: i) Liebermann - Burchard Test: The test solution is treated with 2 ml of chloroform, 3 drops
anhydride and 2 drops of concentrated sulphuric acid. No change is observed which shows the absence of steroids.
Anti- Bacterial studies using Cissus quadrangularis Page 26 b) Test for Carbohydrates : i) Molisch's Test: A portion of test solution is

76% **MATCHING BLOCK 12/17** W

treated with 3 drops of 1% alcoholic α-naphthol and 2ml of concentrated sulphuric acid

is added along the sides of the test tube. Formation of purple colouration at the junction of two liquids shows the presence of carbohydrates. ii) Fehling's Test: A portion of the test solution is trea
volumes of Fehling's solution A and B and then heated in a water bath. Formation of red Cu 2 O precipitate shows the presence of reducing sugars. iii) Tollen's Reagent Test: A portion of the test
treated with equal volumes of Tollens reagents A and B and heated in a water bath. Appearance of silver mirror along the sides of the test tube shows the presence of reducing sugars. iv) Barfoe
portion of test solution 2 ml of Barfoed's reagent is added and heated in a water bath. Formation of red precipitate shows the presence of reducing sugars. c) Test for alkaloids: i) Mayer's Test: A
test solution is shaken with 2N HCl. Aqueous layer formed is decanted to which one or two drops of Mayer's reagent is added. Formation of white turbidity or precipitate shows the presence of
Anti- Bacterial studies using Cissus quadrangularis Page 27 ii) Dragendorff's Test: To a portion of test solution, 2ml of Dragendroff's added. Formation of orange - brown precipitate indicates the
alkaloids. iii) Wagner's Test: To a portion of test solution, 2ml of Wagner's reagent is added. Formation of reddish brown precipitate indicates the presence of alkaloids. iv) Hager's Test: To a portio
solution, 2ml of Hager's reagent is added. Formation of yellow precipitate shows the presence of alkaloids. d) Test for phenolic compounds: i) Neutral Ferric chloride Test: To a portion of test sol
neutral ferric chloride is added. Formation of intense blue colour shows the presence of phenolic compounds. e) Test for saponins: A portion of the test solution is shakes well with 5ml of water
foamy lather shows the presence of saponins. f) Test for xantho Proteins: To a portion of test solution 2ml of concentrated nitric acid is added followed by the addition of excess of liquor ammo
orange precipitate or coloration shows the Absence of xantho proteins.
Anti- Bacterial studies using Cissus quadrangularis Page 28 g) Test for Tannins: i) Lead acetate Test: Water soluble portion of the test solution is treated with basic lead acetate solution Formation
precipitate shows the presence of tannin. h) Test for flavonoids: To a portion of the test solution a small quantity of magnesium powder and 3ml of concentrated hydrochloric acid are added, he
bath. Then the test tube is cooled in running water. Formation of orange colour shows the presence of flavonoids. i) Test for anthraquinone: A portion of the test solution is treated with magnesi
solution. No pink colour shows the absence of anthraquinones. 4.5 PACKING THE SILICA GEL CHROMATOGRAPHY COLUMN Obtain a Pasteur pipette and plug it with a small amount of cotton
lightly into the bottom of the column. Take care that you do not use either too much cotton or pack it too tightly. It is mandatory to prevent the adsorbent from leaking out.
Anti- Bacterial studies using Cissus quadrangularis Page 29 Using a 10 ml beaker gently add a layer of silica to the Pasteur pipette until it is just below the indent in the pipette. Tap the pipette to
level the top of the silica gel and to gently dislodge any trapped air bubbles. When properly packed, the silica gel fills the column to just below the indent on the pipette. This leaves a space of 4-
of the adsorbent for the addition of solvent. Clamp the filled column securely to a ring stand using a small 3-pronged clamp. Pre-Elute the Silica Gel Chromatography Column Using another Pas
add ethyl acetate and petroleum ether to the top of the silica gel in the chromatography column. Monitor the solvent level, both as it flows through the silica gel and the level at the top. Place th
on top of the column, squeeze the bulb, and then remove the bulb while it is still squeezed. Allow the pipette bulb to expand before you remove it from the column, or you will draw solvent and
the bulb. When the bottom solvent level is at the bottom of the column, the pre-elution process is completed and the column is ready to load. Loading the sample into the Silica-Gel Chromato
Column. Mixed about 5 gm of the mixture in a small amount of silica .Add the solution to the prepared chromatography silica gel column via funnel.
Anti- Bacterial studies using Cissus quadrangularis Page 30 Adding the Eluting Solvent to the Silica Gel Chromatography Column Collect 10 ml of the eluting solvent 1:1 ethyl acetate and petrole
Using a pipette add the ethyl acetate and petroleum ether in small portions to the column. Use the pipette bulb to force the ethyl acetate and petroleum ether down through the column. Under
conditions the non-polar component of your mixture will elute from the column. Collect the non-polar component in a pre-weighted 100ml beaker. To collect the polar component you would
the column with a 6:4 ratio of ethyl acetate and petroleum ether. Collect this in another pre-weighted 100 ml beaker. To both beakers containing the polar and non-polar components add a bo
evaporate to dryness by gently heating on a hot plate in the fume hood. Allow both beakers to cool to room temperature before weighting the beakers to find the mass of the two components
difference. Also take the melting point of each component and compare it to it's exactly value. Calculate the weight percentage of polar and non-polar components in the mixture.
Anti- Bacterial studies using Cissus quadrangularis Page 31 RESULTS AND DISCUSSION

Anti- Bacterial studies using Cissus quadrangularis Page 32 CHAPTER V RESULTS AND DISCUSSION 5.1 ETHANOL EXTRACT OF Cissus quadrangularis: The compounds present in ethanol extrac quadrangularis root is tabulated in Table 1. It is observed that the carbohydrates, steroids, phenolic compounds, triterpenoids, alkaloids, saponins, tannins, flavonoids are present in the ethanol ex root.
Anti- Bacterial studies using Cissus quadrangularis Page 33 Fig 8. The anti - bacterial activity of Cissus quadrangularis against different pathogens
Anti- Bacterial studies using Cissus quadrangularis Page 34 Table 2 Zone of inhibition (mm) of Cissus quadrangularis
Page 35 CONCLUSION
Page 36 CHAPTER VI CONCLUSION Presence of sterols was detected in all the extracts through phyto-chemical screening. The methanolic, ethanolic and aqueous extracts showed the presenc and flavanoids, whereas the acetone and aqueous extracts showed the presence of alkaloids. Antibacterial activity of the concerned extracts was comparatively studied against standard drug usi microorganisms (B. subtilis, S. aureus, P. aeruginosa and E. coli). Extracts of petroleum ether, chloroform, acetone, methanol, aqueous and standard drug were chosen for the comparative study, zones of inhibition of diameter 9 mm, 12 mm, 13 mm, 18 mm, 11 mm and 18 mm respectively in culture of S. aureus. The same extracts and standard drug exhibited zones of inhibition of 10 mm mm, 16 mm, 12 mm and 17 mm diameter respectively in case of culture of B. subtilis; zones of 10 mm, 14 mm, 14 mm, 18 mm, 13 mm and 16 mm shown correspondingly in case of culture of E. mm, 12 mm, 13 mm, 18 mm, 11 mm and 19 mm for the same order for culture of P. aeruginosa.
Page 37 REFERENCES
Page 38 CHAPTER VII REFERENCES 1.

100% **MATCHING BLOCK 10/17** W

Nair, Milliken, W., Albert, B., (2004). The use of medicinal plants by the Yanomami Indians of Brazil, Part II. Eco. Botany, 51, 264–278. 2.

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Jainu M. and Devi CS., (2004). "Effect of Cissus quadrangularis on gastric mucosal defensive factors in experimentally induced gastric ulcer- a comparative study with Sucralfate". J. of medi. food 376. 3.

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INTRODUCTION Medicinal Plants have been used as a source of medicine since the dav civilization. The plant designed as medicinal is implied that it is useful as a drug or therap or an active ingredient of a medicinal preparation. Various medicinal plants have been ap years in daily life to treat disease all over the world (Nair et al., 2004). The stem contains unsymmetrical tetracyclic triterpenoids, and two steroidal principles. The presence of β-amyrin, δ-amyrone, and flavonoids (quercetin) having different potential metabolic and effects have also been reported (Jainu and Devi, 2004). The ulcer protective effect of a r extract of C. quadrangularis was similar to that of the reference medicine sucralfate (Jair 2004). Many imitate agents such as estrogens in hormone replacement therapy, especia receptor modulators have been designed to treat osteoporosis but each one of them is side effects such

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as hypercalcemia, hypercalciurea, raised risk of endometrial, and breast cancer, breast tenderness, menstruation, thromboembolic events, vaginal bleeding and hot flushes 3 .

as hypercalcemia, hypercalciurea, raised risk of endometrial, and breast cancer, breast te menstruation, thromboembolic events, vaginal bleeding and hot flushes (

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The use of plant extracts and phytochemicals, with known antibacterial properties, may be of immense importance in therapeutic treatments.

The use of plant extracts and phytochemicals, with known antibacterial properties, may immense importance in therapeutic treatments.

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Kingdom: Plantae Clade: Tracheophytes Clade: Angiosperms Clade: Eudicots Clade: Rosids Order: Vitales Family: Vitaceae Genus: Cissus Species:

Kingdom: Plantae Clade: Tracheophytes Clade: Angiosperms Clade: Eudicots Clade: Ros Vitales Family: Vitaceae Subfamily: Vitoideae Genus: Cissus L.[1] Species

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It is a fleshy, cactus-like climber widely used as a common food item in India. This plant

It is a fleshy, cactus-like liana widely used as a common food item in India. The plant

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helminthiasis, anorexia, dyspepsia, colic, flatulence, skin diseases, leprosy, hemorrhage, epilepsy, convulsion, haemoptysis, tumors, chronic ulcers, swellings.

helminthiasis, anorexia, dyspepsia, colic, flatulence, skin diseases, leprosy, hemorrhage, convulsion, haemoptysis, tumors, chronic ulcers, swellings.

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Extract of the plant prepared by using alcohol, intramuscularly facilitates rapid healing of fracture in albino rats 14 95% Ethanol extract

extract of the plant was locally as well as intramuscularly facilitates rapid healing of fract rats [34]. Ethanol extract (95%)

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The roots and stems are most useful for healing of fracture of the bones. The

The roots and stem are most useful for healing of fracture of the bones. The

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treated with 3 drops of 1% alcoholic α-naphthol and 2ml of concentrated sulphuric acid

treated with 2-3 drops of 1% alcoholic alpha napthol and 2ml of concentrated sulphuric

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ANTIMICROBIAL ACTIVITIES USING CISSUS QUADRANGULARIS PLANT ROOT EXTRACT A Project work submitted to Sadakathullah Appa College (Autonomous), (Re-accredited with "A" Grade b
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Under the Guidance of Dr. A. SYED MOHAMED DEPARTMENT OF CHEMISTRY SADAKATHULLAH APPA COLLEGE (Autonomous) TIRUNELVELI – 11 MAY 2020
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CONTENTS 1. CHAPTER -1 INTRODUCTION 1.1 Plant Morphology 2. CHAPTER – 2 AIMS AND OBJECTIVES 2.1 Aim 2.2 Objectives 3. CHAPTER- 3 REVIEW OF LITRATURE 3.1 Different names of pharmacological activities of Cissus quadrangularis stem 3.3 Secondary Metabolites of Cissus quadrangularis 4. CHAPTER- 4 MATERIALS AND METHODS 4.1 Anti-bacterial activity 4.2 Antimicrob method) 4.2.1 Taxonomial classification of Escherichia coli 4.2.2 Taxonomical classification of Shigella flexneri
4.2.3 Taxonomical classification of Pseudomonas aeroginosa 4.2.4 Taxonomical classification of Staphylococcus 4.3 Preparation of plant extracts 4.4 Preliminary phytochemical analysis 4.5 Pack chromatography column 5. CHAPTER-5 RESULTS AND DISCUSSION 5.1 Ethanol extract of Cissus quadrangularis 6. CHAPTER-6 CONCLUSION 7. CHAPTER-7 REFERENCES
Anti- Bacterial studies using Cissus quadrangularis Page 1 INTRODUCTION
Anti- Bacterial studies using Cissus quadrangularis Page 2 CHAPTER I INTRODUCTION INTRODUCTION Medicinal plants would be the best source for obtaining a variety of drugs. Therefore, su investigated to know about the structural, functional properties and their efficiency of various parts 1 . Herbal treatment is one possible way to treat certain diseases which are caused by certain

65% **MATCHING BLOCK 1/26** W

The use of plant extracts and phytochemicals, with known antibacterial properties, may be of immense importance in therapeutic treatments.

India is rich in various medicinal plants and also familiar for its traditional medicinal systems—Ayurveda, Siddha, and Unani. Ancient Indian medicinal system of health care focused on views of ma pointed out that the healthy human means metabolically well-balanced human beings. They offered programs to rejuvenate the body through diet and nutrition and also treatment methods to such as food allergies, which have few modern treatments. In India, around 20,000 medicinal plants have been documented. The beginning of modern drug research in India can be traced to ea century. Colonel Ram Nath Chopra is recognized as parent of pharmacology, pioneer of systematic studies of indigenous drugs, promoter of Indian systems of medicine,
Anti- Bacterial studies using Cissus quadrangularis Page 3 and patron of pharmacy. The lead given by Chopra led to start of investigation on indigenous drug plants in India. The twentieth centur time in the history of chemistry, during this time synthetic chemistry dominated pharmaceutical industry where natural extract were replaced by the synthetic molecules that often had no conne of these products will soon counterpart predictable pharmaceuticals in the treatment, prevention and diagnosis, while at the same time adding value to agriculture 3 . At twenty first century, exc 11% of the drugs were obtained which are considered as basic and essential. Herbs played an important role in traditional medicine 4 . Alkaloids, flavonoids, tannins, and phenolic compounds are important biologically active constituents of plants 5 . In recent years, attention has been directed in utilizing natural antioxidants substantially (Shahidi et al., 2006; Rajan et al., 2009). One such i Cissus quadrangularis, the parts of these plants have lot of medicinal properties, which are traditionally used by people.
Anti- Bacterial studies using Cissus quadrangularis Page 4 Cissus quadrangularis: 1.1 Plant Morphology:

90% **MATCHING BLOCK 2/26** W

Kingdom: Plantae Clade: Tracheophytes Clade: Angiosperms Clade: Eudicots Clade: Rosids Order: Vitales Family: Vitaceae Genus: Cissus Species: C. quadrangularis Fig. 1 Cissus quadrangularis

Anti- Bacterial studies using Cissus quadrangularis Page 5 Cissus quadrangularis belongs to the vitaceae family and is found in South East Asia.

83% **MATCHING BLOCK 3/26** W

It is a fleshy, cactus-like climber widely used as a common food item in India. This plant

has tri terpenoids, steroids 6,7 stilbenes 8 , flavanoids lipids and several catalpols 9 . The plant which has specific bone fracture healing properties which was approved by ancient Ayurvedic litera

72% **MATCHING BLOCK 4/26** W

The plant is also useful in the treatment of helminthiasis, anorexia, dyspepsia, colic, flatulence, skin diseases, leprosy, hemorrhage, epilepsy, convulsion, haemoptysis, tumors, chronic ulcers, swe

Fig .2 Root of Cissus quadrangularis
Anti- Bacterial studies using Cissus quadrangularis Page 6

95% **MATCHING BLOCK 12/26** W

The roots and stems are most useful for healing of fracture of the bones. The stem is given internally and applied topically in broken bones, used in complaints of the back and spine 10 .

Anti- Bacterial studies using Cissus quadrangularis Page 7 AIMS AND OBJECTIVES
Anti- Bacterial studies using Cissus quadrangularis Page 8 CHAPTER- II AIMS AND OBJECTIVES 2.1 AIM: To prepare the extract of Cissus quadrangularis root with using of ethanol. 2.2 OBJECTIV from the root of Cissus quadrangularis using solvent as ethanol. 2. To analyse the phytochemical component in the root extract of Cissus quadrangularis. 3. To study the anti-bacterial activity of extract. 4. To separate the chemical compounds using column chromatography. The main purpose of this study was to investigate the effect of various conditions including humidity, contact tir and temperature on the adsorption of toluene vapor. Also, in the current study, the antimicrobial potential of ethanolic extract of Cissus quadrangularis roots was investigated.
Anti- Bacterial studies using Cissus quadrangularis Page 9 REVIEW OF LITRATURE
Anti- Bacterial studies using Cissus quadrangularis Page 10 CHAPTER - III REVIEW OF LITRATURE 3.1 Different names of Cissus quadrangularis: LANGUAGE NAMES Marathi Ghanasvel , Ghonuska Harajora Sanskrit Asthibhanga , Asthisamhara, Chaturdharin Chitrakandali, Kandalata, Vajravalli Bengali Harajora Telugu Gudametige , Nalleruvajravalli Gujarati Asthisrnkhala (or) Hadasankal Tamil வச்சிரவல்லி
Anti- Bacterial studies using Cissus quadrangularis Page 11 3.2 SOME PHARMACOLOGICAL ACTIVITIES OF CISSUS QUADRANGULARIS ROOT Antioxidant and free radical scavenging activity: Cis carotene, exhibits strong antioxidant and free radical scavenging activity can be explained with the help of methanol extract of the plant 11, 12 .

93% **MATCHING BLOCK 5/26** W

Antibacterial activity: Methanol extract (90%) and dichloromethane possess antibacterial activity against S. aureus, E. coli, and P. aeruginosa and mutagenicity against Salmonella microsome 13 .

63% **MATCHING BLOCK 6/26** W

Bone healing activity: Extract of the plant prepared by using alcohol, intramuscularly facilitates rapid healing of fracture in albino rats 14 95% Ethanol extract

of the plant possess antiosteoporotic activity. Anti-ulcer activity: Methanol extract produce of the plant shows

94% **MATCHING BLOCK 7/26** W

healing effect on aspirin induced gastric mucosal damage through its antioxidative mechanism 15 . Triterpenoids and β-sitosterol present in

the plant methanol extract possess anti-lipid peroxidating effect and used to

100% **MATCHING BLOCK 8/26** W

prevent gastric damage 16 . Analgesic, anti-inflammatory and stimulatory activity: Methanol extract

of the plant contains flavonoids especially luteolin and by β-sitosterol, which are capable of showing the following activities such as

100% **MATCHING BLOCK 9/26** W

analgesic, anti-inflammatory and venotonic effects associated with hemorrhoids, anti-inflammatory 17 .

Anti- Bacterial studies using Cissus quadrangularis Page 12

84% **MATCHING BLOCK 10/26** W

Toxicology: Cissus quadrangularis extract does not show any toxic effect on oral administration.

The drug prepared by using this plant

100% **MATCHING BLOCK 11/26** W

is safe even at higher dose for a prolonged duration of treatment

was confirmed by Toxicological evaluation of the plant. 3.3 Secondary Metabolites of Cissus quadrangularis Ethanol extract of Cissus quadrangularis used to screen all phytocomponents by usin
revealed the presence of some of the medicinally valuable compounds like Asarone, Phytol, Phenol (Sathyaprabha et al, 2010). Methanol extract of Cissus quadrangularis showed three known c
?- sitosterol (Rao etal.,2011). Asarone Phytol
Anti- Bacterial studies using Cissus quadrangularis Page 13 Phenol Lupeol Freidalin freidalin upeol ?-sitosterol
Anti- Bacterial studies using Cissus quadrangularis Page 14 MATERIALS AND METHODS
Anti- Bacterial studies using Cissus quadrangularis Page 15 CHAPTER IV MATERIALS AND METHODS 4.1 Anti-bacterial activity The anti-microbial activity was analyzed by Disc Diffusion Method.
simple and reliable test to find out the effect of a particular substance on a specific bacterium. Microorganisms used: The antimicrobial activity was tested against the gram positive and gram neg
Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus. 4.2 Antimicrobial activity test (Disk diffusion method): The disk diffusion test, or agar diffusion test, or Kirby–Bauer te
susceptibility test, disc-diffusion antibiotic sensitivity test, KB test), is a test of the antibiotic sensitivity of bacteria. It uses antibiotic discs to test the extent to which bacteria are affected by those a
containing antibiotics are placed on an agar plate where bacteria have been placed, and the plate is left to incubate. If an antibiotic stops the bacteria from growing or kills the
Anti- Bacterial studies using Cissus quadrangularis Page 16 bacteria, there will be an area around the wafer where the bacteria have not grown enough to be visible. This is called a zone of inhibi
Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus were prepared. 2. Nutrient agar plates were prepared and kept for drying. 3. Pathogens were taken in a sterile swab. 4
incorporated separately in separate nutrient agar plate. 5. Sterile disk (6 mm) were placed in the agar plates. 6. 10 µl of samples were impregnated into the sterile disk, control were mentioned us
incubation at 37 °C. 8. After 24 hours the plates were taken and zone of inhibition were measured using ruler. Bacteria used: ? Escherichia coli ? Psedomonas aeroginosa ? Shigella flexneri ? Stap
Pseudomonas fluorescence
Anti- Bacterial studies using Cissus quadrangularis Page 17 4.2.1 Taxonomial classification of Escherichia coli Kingdom Bacteria Phylum Proteobacterea Class Gammaproteobacteria Order Enterc
Enterobacteraceae Genus Escherichia Characteristic features of Escherichia coli: Escherichia coli (commonly abbreviated E.coli) is a Gram-negative, facultatively anaerobic, rod shaped bacterium
the lower intestine of warm-blooded organisms (endotherms). Most E.coli strains are harmless, but some serotypes can cause serious food poisoning recalls due to food contamination. The har
normal flora of the gut and can benefit their hosts by producing vitamin K2, and preventing colonization of the intestine with pathogenic bacteria. E.coli and other facultative constitute about 0.1
transmission is the major route through which pathogenic strains of the bacterium cause disease. Cells are able to survive outside the body for
Anti- Bacterial studies using Cissus quadrangularis Page 18 a limited amount of time, which makes them ideal indicator organisms to test environmental samples for faecal contamination. There
research that has examined environmentally persistent E.coli which can survive for extended periods outside of the host. 4.2.2 Taxonomical classification of Shigella flexneri Kingdom Bacteria Do
Proteobacteria Class Gammaproteobacteria Order Enterobacterales Family Enterobacteriaceae Genus Shigella Species S. flexneri Characteristics features of S. flexneri Shigella flexneri is a specie
the genus Shigella. Shigella flexneri is a rod shaped, nonflagellar bacterium that relies on actin-based motility. S. flexneri is an intracellular bacterium that infects the epithelial lining of the mamm
bacterium is acid tolerant and can survive conditions of pH 2.
Anti- Bacterial studies using Cissus quadrangularis Page 19 4.2.3 Taxonomical classification of Pseudomonas aeroginosa: Kingdom Bacteria Phylum Proteobacterea Class Gammaproteobacteria
Family Pseudomonadaceae Genus Pseudomonas Characteristics features of Pseudomonas aerogenosa Pseudomonas aeroginosa is a common Gram-negative rod-shaped bacterium that can c
animals, including humans. A species of considerable medical importance, P.aeroginosa is a prototypical "multidrug resistance (MRD) pathogen" that is recognized for its ubiquity, its intrinsically a
mechanisms, and its association with serious illness, especially nosocomical infections such as ventilator, associated pneumonia and various sepsis sundromes. The organism is considered oppo
infection is often superimposed upon acute or chronic morbidity, most notably cystic fibrosis and traumatic burns – or fund in immuno-compromised individuals, but the
Anti- Bacterial studies using Cissus quadrangularis Page 20 organism does produce a range of clinically important infections in the immune- component and in situations where no pre-existing
tub folliculitis. 4.2.4 Taxonomical classification of staphylococcus: Kingdom Bacteria Phylum Firmicutes Class Bacilli Order Bacillales Family Staphylococcaceae Genus Staphylococcus Character
The genus is named by bacteriologist Alexander Ogston in 1882. It is derived from the Latin word meaning bunch of grapes in spherical bacterium. They include at least 40 species. Two sub spec
three subspecies in one species and four subspecies in one species. In these, most of them are harmless. They have a cell wall structure and G+C content of DNA in a range of 30-40 %. They gr
Anti- Bacterial studies using Cissus quadrangularis Page 21 The genus staphylococcus colonizes the skin and upper respiratory tracts of mammals and birds. It can cause a wide variety of disease
through either toxin production or penetration. They are common causes of food poisoning, they can be produced by bacteria growing in improperly stored food items. The most common sial
staphylococci, as bacterial infections. Antibiotics is basically used. They are commonly cephalosporins, nafcillin, sulfadurgsvancomycin. Vancomycin is highly used. 4.2.5 Taxonomical classificatio
fluorescence: Kingdom Bacteria Domain Bacteria Phylum Proteobacteria Class Gammaproteobacteria Order Pseudomonadales Family Pseudomonadaceae Genus Pseudomonas Species group
group Species: P. fluorescens Pseudomonas fluorescens is a common Gram-negative, rod- shaped bacterium. It belongs to the Pseudomonas genus. It has an extremely
Anti- Bacterial studies using Cissus quadrangularis Page 22 versatile metabolism, and can be found in the soil and in water. Optimal temperatures for growth of P. fluorescens are 25–30°C. It test
is also a nonsaccharolytic bacterial species.
Anti- Bacterial studies using Cissus quadrangularis Page 23 Fig 3. Laminar Air Flow Chamber used for Microorganism culturing Fig 4.Preparation of agar nutrient
Anti- Bacterial studies using Cissus quadrangularis Page 24 Fig 5. Prepared Nutrient Medium Fig 6.Solidified form of agar in petri dish
Page 25 Fig 7. Preparation for Disc Diffusion Method
Page 26 4.3 Preparation of plant extracts: Fresh root samples Cissus quadrangularis were washed under running tap water and dried for 48 hrs in a hot air oven at 60°C. Dried root sample were g
blender to obtain a fine powder and stored in polythene bags until needed for analysis. 30 g of the sample is soaked in the water. The solution was vigorously shaken at room temperature for 48
Whatmann No.1 filter paper. The filtrate is used for the phytochemical analysis. Chemical tests for screening and identification of bioactive chemical constituents in the Cissus quadrangularis spe
using the standard procedures. 4.4 Preliminary phytochemical analysis : 5g of the air dried, powdered roots of Cissus quadrangularis was successively extracted with petroleum ether (40-60 ? C)
chloroform. All these extracts were subjected to preliminary phytochemical analysis. The following tests were carried out. The results are presented in Table 3. a)Test for steroids: i) Liebermann -
is treated with 2 ml of chloroform, 3 drops acetic anhydride and 2 drops of concentrated sulphuric acid. No change is observed which shows the absence of steroids.
Page 27 b) Test for Carbohydrates : i) Molisch's Test: A portion of test solution is
treated with 3 drops of 1% alcoholic α-naphthol

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and 2ml of concentrated sulphuric acid is added along the sides of the test tube. Formation of purple colouration at the

junction of two liquids shows the presence of carbohydrates. ii) Fehling's Test: A portion of the test solution is treated

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with equal volumes of Fehling's solution A and B and then heated in a water bath.

Formation of red Cu 2 O precipitate shows the presence of reducing sugars. iii) Tollen's Reagent Test: A portion of the test solution is treated

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with equal volumes of Tollens reagents A and B and heated in a water bath.

Appearance of silver mirror along the sides of the test tube shows the presence of reducing sugars. iv) Barfoed's Test: To a portion of test solution 2 ml of Barfoed's reagent is added and heated i red precipitate shows the presence of reducing sugars. c) Test for alkaloids: i) Mayer's Test: A portion of the test solution is shaken with 2N HCl. Aqueous layer formed is decanted to which one d is added. Formation of white turbidity or precipitate shows the presence of alkaloids.
Page 28 ii) Dragendorff's Test: To a portion of test solution, 2ml of Dragendroff's added. Formation of orange - brown precipitate indicates the presence of alkaloids. iii) Wagner's Test: To a portic Wagner's reagent is added. Formation of reddish brown precipitate indicates the presence of alkaloids. iv) Hager's Test: To a portion of test solution, 2ml of Hager's reagent is added. Formation o presence of alkaloids. d) Test for phenolic compounds: i) Neutral Ferric chloride Test: To a portion of test solution, 1 ml of neutral ferric chloride is added. Formation of intense blue colour shows compounds. e) Test for saponins: A portion of the test solution is shakes well with 5ml of water Formation of foamy lather shows the presence of saponins. f) Test for xantho Proteins: To a portic concentrated nitric acid is added followed by the addition of excess of liquor ammonia, No reddish orange precipitate or coloration shows the Absence of xantho proteins.
Page 29 g) Test for Tannins: i) Lead acetate Test: Water soluble portion of the test solution is treated with basic lead acetate solution Formation of white precipitate shows the presence of tannin. portion of the test solution a small quantity of magnesium powder and 3ml of concentrated hydrochloric acid are added, heated in a water bath. Then the test tube is cooled in running water. Fo shows the presence of flavonoids. i) Test for anthraquinone: A portion of the test solution is treated with magnesium acetate solution. No pink colour shows the absence of anthraquinones. 4.5 F CHROMATOGRAPHY COLUMN Obtain a Pasteur pipette and plug it with a small amount of cotton. Tamp it down lightly into the bottom of the column. Take care that you do not use either too tightly. It is mandatory to prevent the adsorbent from leaking out. Using a 10 ml beaker gently add a layer of silica to the Pasteur pipette until it is just below the indent in the pipette. Tap the pipe top of the silica gel and to gently dislodge any trapped air bubbles. When properly packed, the silica gel fills the column to just below the indent on the pipette. This leaves a space
Page 30 of 4–5 cm on top of the adsorbent for the addition of solvent. Clamp the filled column securely to a ring stand using a small 3-pronged clamp. Pre-Elute the Silica Gel Chromatography pipette add ethyl acetate and petroleum ether to the top of the silica gel in the chromatography column. Monitor the solvent level, both as it flows through the silica gel and the level at the top. the column, squeeze the bulb, and then remove the bulb while it is still squeezed. Allow the pipette bulb to expand before you remove it from the column, or you will draw solvent and silica gel solvent level is at the bottom of the column, the pre-elution process is completed and the column is ready to load. Loading the sample into the Silica-Gel Chromatography Column. Mixed abou amount of silica .Add the solution to the prepared chromatography silica gel column via funnel. Adding the Eluting Solvent to the Silica Gel Chromatography Column Collect 10 ml of the eluting petroleum ether. Using a pipette add the ethyl acetate and petroleum ether in small portions to the column. Use the pipette bulb to force the ethyl acetate and petroleum ether down through th conditions the non-polar component of your mixture will elute from the column.
Page 31 Collect the non-polar component in a pre-weighted 100ml beaker. To collect the polar component you would need to elute the column with a 6:4 ratio of ethyl acetate and petroleum pre-weighted 100 ml beaker. To both beakers containing the polar and non-polar components add a boiling stone and evaporate to dryness by gently heating on a hot plate in the fume hood. A room temperature before weighting the beakers to find the mass of the two components by weight difference. Also take the melting point of each component and compare it to it's exactly valu percentage of polar and non-polar components in the mixture.
Page 32 RESULTS AND DISCUSSION
Page 33 CHAPTER V RESULTS AND DISCUSSION 5.1 ETHANOL EXTRACT OF Cissus quadrangularis: The compounds present in ethanol extract of Cissus quadrangularis root is tabulated in Table carbohydrates, steroids, phenolic compounds, triterpenoids, alkaloids, saponins, tannins, flavonoids are present in the ethanol extract of the root. Table 1: Phytochemical Analysis of Plant Extract Phytochemical constituents Cissus quadrangularis 1. Flavonoid Positive 2. Protein Positive 3. Tannin Positive Positive 4. Alkaloid Positive 5. Phenol Positive 6. Carbohydrate Positive 7. Quinone Po Glycoside Positive 10. Anthraquinone Negative
Page 34 Fig 8. The anti - bacterial activity of Cissus quadrangularis against different pathogens Table 2 Antimicrobial Activity of Cissus quadrangularis Against Selected Pathogens
Page 35 CONCLUSION
Page 36 CHAPTER VI CONCLUSION In the present study phytochemical analysis of the Cissus quadrangularis was carried out to investigate antimicrobial activity. The antibacterial activity tested against Staphylococcus aureus revealed 28mm in diameter which is considered to be the best result with the plant extract. It is followed by Pseudomonas fluorescence and Pseudomonas aerug inhibition of 26 mm in diameter. The plant extract showed a remarkable activity with the above organisms. Our results coincides with the findings of Garima et al., 2009 where in they reported th extracts was found to be inhibitory against both gram positive and gram negative bacterial pathogens. In the present study, antibacterial activity of petroleum ether – chloroform extracts of Cissu The extracts revealed remarkable inhibitory activity against gram positive and gram negative bacterial pathogens. Treatment of diseases possesses challenging problems due to emerging infectio number of multidrug resistance microbial pathogens. In spite of large number of antibiotics and drugs used a substantial need for new class of potential compound as drug is obtained from plan extract play an important group in designing a new class of structural antibiotics of medicinal importance with new mechanism of action.

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These findings clearly demonstrated that the bioactive metabolites present in Cissus quadrangularis can be used for the treatment of disease.

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Page 38 CHAPTER VII REFERENCES 1. Eloff, J.N. (1998); A Sensitive and Quick Microplate Method to Determine the Minimal Inhibitory Concentration of Plant Extracts for Bacteria. Planta Medica S.I.Ebigwei; O.O.Adebawo; F.O.Osiyemi; Plasmid profiles and antibiotic susceptibility patterns of Lactobacillus isolated from fermented foods in Nigeria, Food Microbiology, Volume 10, Issue 4, Au IlyaRaskin et al; Plants and human health in the twenty-first century ; Volume 20, Issue 12, 1 December 2002, Pages 522-531. 4. Shazia Ali ; Singh, B. ; Anshu Dhaka ; Deepak Kumar ; Study on co [Abelmoschus esculentus (L.) Moench.]. Plant Archives 2008 Vol.8 No.1 pp.405-407. 5. S. Sukumaran; SKiruba et al; Phytochemical constituents and antibacterial efficacy of the flowers of Peltop Baker ex Heyne; Asian Pacific Journal of Tropical Medicine Volume 4, Issue 9, September 2011, Pages 735-738. 6.

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1/26 **SUBMITTED TEXT** 20 WORDS **65%** **MATCHING TEXT**

The use of plant extracts and phytochemicals, with known antibacterial properties, may be of immense importance in therapeutic treatments.

The use of plant extracts and phytochemicals, both with known antimicrobial p significance in therapeutic treatments.

W https://ijpsr.com/bft-article/phytochemical-screening-and-antibacterial-activity-of-gymnema-sylve ...

2/26 **SUBMITTED TEXT** 25 WORDS **90%** **MATCHING TEXT**

Kingdom: Plantae Clade: Tracheophytes Clade: Angiosperms Clade: Eudicots Clade: Rosids Order: Vitales Family: Vitaceae Genus: Cissus Species: C. quadrangularis Fig. 1 Cissus quadrangularis

Kingdom: Plantae Clade: Tracheophytes Clade: Angiosperms Clade: Eudicots Cl Family: Vitaceae Genus: Cissus Species: C. quadrangularis Binomial name quadr

W https://en.wikipedia.org/wiki/Cissus_quadrangularis

3/26 **SUBMITTED TEXT** 18 WORDS **83%** **MATCHING TEXT**

It is a fleshy, cactus-like climber widely used as a common food item in India. This plant

It is a fleshy, cactus-like liana widely used as a common food item in India. The

W https://www.researchgate.net/publication/329182388_CISSUS_QUADRANGULARIS_EXTRACT_MEDIATED_GREEN_S ...

4/26 **SUBMITTED TEXT** 39 WORDS **72%** **MATCHING TEXT**

The plant is also useful in the treatment of helminthiasis, anorexia, dyspepsia, colic, flatulence, skin diseases, leprosy, hemorrhage, epilepsy, convulsion, haemoptysis, tumors, chronic ulcers, swellings.

The plant is believed to be useful helminthiasis, anorexia, dyspepsia, colic, flatule hemorrhage, epilepsy, convulsion, haemoptysis, tumors, chronic ulcers, swellin

W https://www.ijppsjournal.com/Vol3Suppl4/2439.pdf

5/26 **SUBMITTED TEXT** 32 WORDS **93%** **MATCHING TEXT**

Antibacterial activity: Methanol extract (90%) and dichloromethane possess antibacterial activity against S. aureus, E. coli, and P. aeruginosa and mutagenicity against Salmonella microsome 13 .

antibacterial activity Methanol extract (90%) and dichloromethane extract of ste activity against S. aureus, E. coli, and P. aeruginosa and mutagenicity against Sal

W https://www.ijppsjournal.com/Vol3Suppl4/2439.pdf

6/26	SUBMITTED TEXT	30 WORDS	63%	MATCHING TEXT
Bone healing activity: Extract of the plant prepared by using alcohol, intramuscularly facilitates rapid healing of fracture in albino rats 14 95% Ethanol extract			Bone healing activity Paste of alcoholic extract of the plant was locally as well a rapid healing of fracture in albino rats. [31] Ethanol extract (95%)	
W https://www.ijppsjournal.com/Vol3Suppl4/2439.pdf				

7/26	SUBMITTED TEXT	23 WORDS	94%	MATCHING TEXT
healing effect on aspirin induced gastric mucosal damage through its antioxidative mechanism 15 . Triterpenoids and β-sitosterol present in			healing effect on aspirin induced gastric mucosal damage in rats through its ant Triterpenoids and β- sitosterol present in	
W https://www.ijppsjournal.com/Vol3Suppl4/2439.pdf				

8/26	SUBMITTED TEXT	19 WORDS		MATCHING TEXT
prevent gastric damage 16 . Analgesic, anti-inflammatory and stimulatory activity: Methanol extract			100% prevent gastric damage. Analgesic, anti-inflammatory and stimulatory activity [3	
W https://www.ijppsjournal.com/Vol3Suppl4/2439.pdf				

9/26	SUBMITTED TEXT	15 WORDS	100%	MATCHING TEXT
analgesic, anti-inflammatory and venotonic effects associated with hemorrhoids, anti-inflammatory 17 .			analgesic, anti-inflammatory and venotonic effects associated with hemorrhoid	
W https://www.ijppsjournal.com/Vol3Suppl4/2439.pdf				

10/26	SUBMITTED TEXT	15 WORDS	84%	MATCHING TEXT
Toxicology: Cissus quadrangularis extract does not show any toxic effect on oral administration.			Toxicology [45] The Cissus quadrangularis extract does not produce any toxic e	
W https://www.ijppsjournal.com/Vol3Suppl4/2439.pdf				

11/26	SUBMITTED TEXT	13 WORDS	100%	MATCHING TEXT
is safe even at higher dose for a prolonged duration of treatment			is safe even at higher dose for a prolonged duration of treatment.	
W https://www.ijppsjournal.com/Vol3Suppl4/2439.pdf				

12/26	SUBMITTED TEXT	48 WORDS	95%	MATCHING TEXT
The roots and stems are most useful for healing of fracture of the bones. The stem is given internally and applied topically in broken bones, used in complaints of the back and spine 10 .			The roots and stems are most useful for healing of fracture of the bones. The st applied topically in broken bones, used in complaints of the back and spine. [20	
W https://www.ijppsjournal.com/Vol3Suppl4/2439.pdf				

13/26	SUBMITTED TEXT	22 WORDS	50%	MATCHING TEXT
and 2ml of concentrated sulphuric acid is added along the sides of the test tube. Formation of purple colouration at the			and 2ml of H 2 was added along the sides of the test tube and the formation of	
W https://www.doc-developpement-durable.org/file/Arbres-Bois-de-Rapport-Reforestation/FICHES_ARBRES ...				

14/26	SUBMITTED TEXT	16 WORDS	66%	MATCHING TEXT
with equal volumes of Tollens reagents A and B and heated in a water bath.			with equal volumes of Fehling's solution A and B and heated in boiling water ba	
W https://www.doc-developpement-durable.org/file/Arbres-Bois-de-Rapport-Reforestation/FICHES_ARBRES ...				

15/26	SUBMITTED TEXT	17 WORDS	82%	MATCHING TEXT
with equal volumes of Fehling's solution A and B and then heated in a water bath.			with equal volumes of Fehling's solution A and B and heated in boiling water ba	
W https://www.doc-developpement-durable.org/file/Arbres-Bois-de-Rapport-Reforestation/FICHES_ARBRES ...				

16/26	SUBMITTED TEXT	31 WORDS	84%	MATCHING TEXT
Jaiswal S, Singh S V, Singh B, Singh HN. Plants used for tissue healing of animals. Natural Products Radiance. 2004; 3: 284-92.			Jaiswal S, Singh SV, Singh B, Singh HN. Plants used for tissue healing of animals. 2004; 3: 284-92. 39.	
W https://www.ijppsjournal.com/Vol3Suppl4/2439.pdf				

17/26 **SUBMITTED TEXT** 41 WORDS **100% MATCHING TEXT**

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Luseba D, Elgorashi EE, Ntloedibe DT, Staden JV. Antibacterial, anti- inflammato some medicinal plants used in South Africa for the treatment of wounds and ret South African Journal of Botany 2007; 73: 378-83. 30.

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18/26 **SUBMITTED TEXT** 27 WORDS **100% MATCHING TEXT**

These findings clearly demonstrated that the bioactive metabolites present in Cissus quadrangularis can be used for the treatment of disease.

These findings clearly demonstrated that the bioactive metabolites present in C used for the treatment of disease. •

W https://www.journalijar.com/article/17095/antimicrobial-activities-of-silver-nanoparticles-synthe ...

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Studies on "Cissus Quadrangularis" in fracture by using phosphorus 32. III

W https://www.researchgate.net/publication/285707665_Pharmacological_and_therapeutic_activity_of_Ci ...

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Role of medicinal plants and natural products on osteoporotic fracture healing.

Role of Medicinal Plants and Natural Products on Osteoporotic Fracture Healing

W https://www.researchgate.net/publication/285707665_Pharmacological_and_therapeutic_activity_of_Ci ...

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Udupa, K.N., Prasad, G. and Sen, S.P., 1965. The effect of phytogenic anabolic st fracture repair. Life

W https://escipub.com/ajcrr-2020-07-1705/

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