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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 Tirunelveli - 11 Affiliated to Manonmaniam Sundaranar University

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In partial fulfillment of the requirement for the degree of BACHELOR OF SCIENCE IN

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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 result of 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 College (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: Tirunelveli
ACKNOWLEDGEMENTS I raise my heart in deep gratitude to THE ALMIGHTY for HIS guidance and good health that He has given me to carry out my dissertation with the attentiveness and passion. I record my heartfelt thanks to my guide Dr. A. SYED MOHAMED, Assistant Professor and Head, Research Department of Chemistry, Sadakathullah Appa College (Autonomous), Tirunelveli for his guidance, valuable suggestions, thought provoking questions with his excellent execution, encouraging attitude, efforts to bring out the best in me and his availability at all time this present study have seen the light of the day. I hereby acknowledge my sincere thanks to our honorable Secretary ALHAJ. T.E.S.FATHU RABBANI, Sadakathullah Appa College (Autonomous), Tirunelveli for his help to do this project work. I wish to express our deep sense of gratitude to our beloved principal Dr. M. MOHAMED SATHIK, Sadakathullah Appa College (Autonomous), Tirunelveli for granting permission for project skillfully. I wish thanks to Dr. S. Mahadevan (Dean of Arts), Dr. S.M. Abdul Kader (Dean of Science), Dr. S.H. Mohamed Ameen (Controller of Examination), Dr. M. Sheik Muhideen Badhusha, Danish, Dr. P. Jeslin Kanaga Inba , Dr. M. Thameem Ansari, Dr. M.A.Sabitha, Dr. Raihana Imran Khan, Dr. S.M.Y Mohamed Muktar Ali, Dr. S. Brillians Revin For their support and help. I extended my thanks to our technical assistant Mr.Feroz Khan for helping me in the Laboratory. I wish my thanks to all members of teaching and non- teaching of Department of Chemistry, Sadakathullah Appa College (Autonomous), Tirunelveli. I remain thankful to all my family members and my friends for their words and encouragement they offered me doing my thesis work.

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 Cissus 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-bacterial activity test (Disk diffusion method) 4.2.1 Taxonomical classification of Escherichia coli 4.2.2 Taxonomical classification of Bacillus subtilis 4.2.3 Taxonomical classification of Pseudomonas aeruginosa 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

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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 or 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 triterpenoids and two steroidal principles. The presence of β -sitosterol, δ -amyryrin, δ -amyryne, and flavonoids (quercetin) having different potential metabolic and physiological effects have also been reported 2 . The 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 and 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 being 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 India, 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 as a pharmacologist, 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 indigenous 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 extracts were replaced 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. In 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 quadrangularis* Plant Morphology:

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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.

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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 literature is also useful in the treatment of

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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

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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, hemorrhoids, 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 lime water and preserved 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 OBJECTIVE: To 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 effect of the 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 conditions 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 *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 , Ghonuskar, Mhasvel Hindi Harajora Sanskrit Asthibhanga , Asthisamhara, Chaturdharin, Chitrakandali, Kandalata, Vajravalli Bengali Harajora Telugu Gudameti , Nalleruvajravalli Gujarati Asthisrnkhala (or) Harajora பிரண்டை (அல்லது) வச்சிராவல்லி

Anti- Bacterial studies using *Cissus quadrangularis* Page 11 3.2 SOME PHARMACOLOGICAL ACTIVITIES OF *CISSUS QUADRANGULARIS* STEM Antioxidant and free radical scavenging activity: *Cissus 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: Ethanol extract 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 fracture

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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

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 activity. 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 activity: 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 activity 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 at a 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* screened all phytochemicals by using Gas Chromatography method revealed the presence of some of the medicinally valuable compounds like Asarone, Phytol, Phenol (Sathyaprabha et al, 2011). Ethanol extract Of the stems of *Cissus quadrangularis* showed three known compounds lupeol, freidalin and β -sitosterol (Rao et al.,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 positive and 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 agar diffusion 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 the sensitivity of bacteria to which 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 antibiotic kills 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 inhibition. 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 swabs. 4. Each swab was 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 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* ? *Pseudomonas aeruginosa* ? *Bacillus subtilis* ? *Staphylococcus*

Anti- Bacterial studies using *Cissus quadrangularis* Page 17 4.2.1 Taxonomical classification of *Escherichia coli* Kingdom Bacteria Phylum Proteobacteria Class Gammaproteobacteria Order Enterobacteriales Family Enterobacteriaceae Genus *Escherichia* Characteristic features of *Escherichia coli*: *Escherichia coli* (commonly abbreviated *E.coli*) is a Gram-negative, facultatively anaerobic, rod shaped bacterium 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 contamination. 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 other facultative constitute about 0.1% of gut flora, and faecal-oral transmission is the major route through which pathogenic strains of the bacterium cause disease. Cells are able to survive outside the host for weeks. 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 is a 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* Kingdom Bacteria Phylum Firmicutes Class Bacilli Order Bacillales Family Bacillaceae Genus *Bacillus* Species *B. subtilis* Characteristics features of *Bacillus subtilis* *Bacillus subtilis* is a Gram-positive, rod-shaped bacterium that is naturally found in soil and vegetation. *Bacillus subtilis* grows in the mesophilic temperature range. The optimal temperature is 25-35 °C (Entre Genome Project). Spores and 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-resistant spores. 4.2.3 Taxonomical classification of *Pseudomonas aeruginosa*: Kingdom Bacteria Phylum Proteobacteria Class Gammaproteobacteria Order Pseudomonadales Family Pseudomonadaceae Genus *Pseudomonas* Characteristics features of *Pseudomonas aeruginosa* *Pseudomonas aeruginosa* is a common Gram-negative rod-shaped bacterium that can cause disease in plants and animals, including humans. A species of considerable medical importance, *P.aeruginosa* is a prototypical "multidrug resistance (MRD) pathogen" that is recognized for its ubiquity, its intrinsically advanced antibiotic resistance mechanisms, and its association with serious illness, especially nosocomial infections such as ventilator-associated pneumonia and various sepsis syndromes.

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 notably in lung fibrosis and traumatic burns – or found in immuno-compromised individuals, but the organism does produce a range of clinically important infections in the immune-competent and in situations where 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 Staphylococcaceae 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 shape. 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. The 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 *Staphylococcus* 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 common causes of 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 basically used are commonly cephalosporins, nafcillin, sulfadiazine, vancomycin. 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 medium

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 hours in an 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 water 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 and 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 the 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 phytochemical 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 of acetic 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%

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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 coloration at the junction of two liquids shows the presence of carbohydrates. ii) Fehling's Test: A portion of the test solution is treated with equal volumes of Fehling's solution A and B and then heated in a water bath. Formation of red Cu_2O precipitate shows the presence of reducing sugars. iii) Tollen's Reagent Test: A portion of the test solution is treated with equal volumes of Tollen's 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: A 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 portion of 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 alkaloids. ii) Dragendorff's Test: To a portion of test solution, 2ml of Dragendorff's reagent is added. Formation of orange-brown precipitate indicates the presence of 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 portion of test 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 solution, neutral ferric chloride is added. Formation of intense blue color shows the presence of phenolic compounds. e) Test for saponins: A portion of the test solution is shaken well with 5ml of water. Frothy 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 ammoniac. Formation of 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 of white 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, heated in a water bath. Then the test tube is cooled in running water. Formation of orange color shows the presence of flavonoids. i) Test for anthraquinone: A portion of the test solution is treated with magnesium solution. No pink color 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 wool. Push it 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 4-5 mm 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 Pasteur 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. Place the pipette 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 silica into 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 Chromatography 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 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 the column. Under these conditions the non-polar component of your mixture will elute from the column. Collect the non-polar component in a pre-weighed 100ml beaker. To collect the polar component you would add the column with a 6:4 ratio of ethyl acetate and petroleum ether. Collect this in another pre-weighed 100 ml beaker. To both beakers containing the polar and non-polar components add a boiling solvent and evaporate to dryness by gently heating on a hot plate in the fume hood. Allow both beakers to cool to room temperature before weighing the beakers to find the mass of the two components. Calculate the difference. Also take the melting point of each component and compare it to its exact 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 extract of *Cissus 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 extract.

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 presence of sterols 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 used against 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, 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. coli*. 10 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.

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<p>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 or an active 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 triterpenoids, and two steroidal principles. The presence of β-sitosterol, δamyryn, δ-amyrone, and flavonoids (quercetin) having different potential metabolic and physiological effects have also been reported 2 . The ulcer protective effect of a methanolic extract of <i>C. quadrangularis</i> was similar to that of the reference medicine sucralfate 2 . Many imitate agents such as estrogens in hormone replacement therapy, especially estrogen receptor modulators have been designed to treat osteoporosis but each one of them is linked with side effects such</p>		<p>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 or an active ingredient of a medicinal preparation. Various medicinal plants have been applied for 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-amyryn, δ-amyrone, and flavonoids (quercetin) having different potential metabolic and physiological effects have also been reported (Jainu and Devi, 2004). The ulcer protective effect of a methanolic extract of <i>C. quadrangularis</i> was similar to that of the reference medicine sucralfate (Jainu and Devi, 2004). Many imitate agents such as estrogens in hormone replacement therapy, especially estrogen receptor modulators have been designed to treat osteoporosis but each one of them is linked with side effects such</p>	
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<p>as hypercalcemia, hypercalciurea, raised risk of endometrial, and breast cancer, breast tenderness, menstruation, thromboembolic events, vaginal bleeding and hot flushes 3 .</p>		<p>as hypercalcemia, hypercalciurea, raised risk of endometrial, and breast cancer, breast tenderness, menstruation, thromboembolic events, vaginal bleeding and hot flushes (</p>	
<p>W https://www.researchgate.net/publication/340489067_Antibacterial_Activity_of_Cissus_quadrangularis...</p>			
4/17	SUBMITTED TEXT	20 WORDS	100% MATCHING TEXT
<p>The use of plant extracts and phytochemicals, with known antibacterial properties, may be of immense importance in therapeutic treatments.</p>		<p>The use of plant extracts and phytochemicals, with known antibacterial properties, may be of immense importance in therapeutic treatments.</p>	
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<p>Kingdom: Plantae Clade: Tracheophytes Clade: Angiosperms Clade: Eudicots Clade: Rosids Order: Vitales Family: Vitaceae Genus: <i>Cissus</i> Species:</p>		<p>Kingdom: Plantae Clade: Tracheophytes Clade: Angiosperms Clade: Eudicots Clade: Rosids Order: Vitales Family: Vitaceae Subfamily: Vitoideae Genus: <i>Cissus</i> L.[1] Species</p>	
<p>W https://en.wikipedia.org/wiki/Cissus</p>			
6/17	SUBMITTED TEXT	18 WORDS	83% MATCHING TEXT
<p>It is a fleshy, cactus-like climber widely used as a common food item in India. This plant</p>		<p>It is a fleshy, cactus-like liana widely used as a common food item in India. The plant</p>	
<p>W https://www.researchgate.net/publication/242418266_Cissus_quadrangularis_plant_extract_enhances_t...</p>			
7/17	SUBMITTED TEXT	30 WORDS	100% MATCHING TEXT
<p>helminthiasis, anorexia, dyspepsia, colic, flatulence, skin diseases, leprosy, hemorrhage, epilepsy, convulsion, haemoptysis, tumors, chronic ulcers, swellings.</p>		<p>helminthiasis, anorexia, dyspepsia, colic, flatulence, skin diseases, leprosy, hemorrhage, convulsion, haemoptysis, tumors, chronic ulcers, swellings.</p>	
<p>W https://www.researchgate.net/publication/242418266_Cissus_quadrangularis_plant_extract_enhances_t...</p>			
8/17	SUBMITTED TEXT	27 WORDS	65% MATCHING TEXT
<p>Extract of the plant prepared by using alcohol, intramuscularly facilitates rapid healing of fracture in albino rats 14 95% Ethanol extract</p>		<p>extract of the plant was locally as well as intramuscularly facilitates rapid healing of fracture in albino rats [34]. Ethanol extract (95%)</p>	
<p>W https://www.researchgate.net/publication/242418266_Cissus_quadrangularis_plant_extract_enhances_t...</p>			
9/17	SUBMITTED TEXT	16 WORDS	90% MATCHING TEXT
<p>The roots and stems are most useful for healing of fracture of the bones. The</p>		<p>The roots and stem are most useful for healing of fracture of the bones. The</p>	
<p>W https://www.researchgate.net/publication/242418266_Cissus_quadrangularis_plant_extract_enhances_t...</p>			
10/17	SUBMITTED TEXT	25 WORDS	100% MATCHING TEXT
<p>Nair, Milliken, W., Albert, B., (2004). The use of medicinal plants by the Yanomami Indians of Brazil, Part II. <i>Eco. Botany</i>, 51, 264–278. 2.</p>		<p>Nair, Milliken, W., Albert, B., (2004). The use of medicinal plants by the Yanomami Indians of Brazil, Part II. <i>Eco. Botany</i>, 51, 264–278. 14.</p>	
<p>W https://www.researchgate.net/publication/340489067_Antibacterial_Activity_of_Cissus_quadrangularis...</p>			

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<p>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, 7(3), 372-376. 3.</p> <p>W https://www.researchgate.net/publication/340489067_Antibacterial_Activity_of_Cissus_quadrangulari ...</p>		<p>Jainu M. and Devi CS., (2004). "Effect of Cissus quadrangularis on gastric mucosal defen in experimentally induced gastric ulcer- a comparative study with of medi. food, 7(3), 372-376. 3.</p>	
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<p>treated with 3 drops of 1% alcoholic α-naphthol and 2ml of concentrated sulphuric acid</p> <p>W https://www.researchgate.net/profile/Manikandan_p4/publication/328701401_FULL_THESIS/links/5bdc85 ...</p>		<p>treated with 2-3 drops of 1% alcoholic alpha naphthol and 2ml of concentrated sulphuric acid</p>	
13/17	SUBMITTED TEXT	29 WORDS	100% MATCHING TEXT
<p>Ogey A., Bayraktar F., Sevin G., (2001). A comparative study of Raloxifen and estrogen on bone strength and cholesterol levels in ovariectomized rats, End. Abstracts, 3, 10. 4.</p> <p>W https://www.researchgate.net/publication/340489067_Antibacterial_Activity_of_Cissus_quadrangulari ...</p>		<p>Ogey A., Bayraktar F., Sevin G., (2001). A comparative study of Raloxifen and estrogen on strength and cholesterol levels in ovariectomized rats, End. Abstracts, 3, 10. 16.</p>	
14/17	SUBMITTED TEXT	18 WORDS	82% MATCHING TEXT
<p>Adesanya SA, Nia R, Martin MT, Boukamcha N, Montagnac A, Pais M. Stilbene derivatives from Cissus quadrangularis.</p> <p>W https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5115982/</p>		<p>Adesanya SA, Nia R, Martin M, Boukamcha N, Montagnac A, Pas M. Stilbene derivatives from Cissus quadrangularis.</p>	
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<p>Mallika J, Shyamala CSD, In vitro and In vivo evaluation of free radical scavenging potential of Cissus quadrangularis. Afri J of Biomed Res, 2005, 8, 95-99. 12. Mehta M, Kaur N, Bhutani K., Determination of marker constituents from</p> <p>W https://ijpsr.com/bft-article/pharmacognostic-evaluation-of-cissus-quadrangularis-l-stem/?view=full-text</p>		<p>Mallika J, Shyamala CSD: In vitro and In vivo evaluation of free radical scavenging potential of Cissus quadrangularis . Afri J of Biomed Res 2005; 8: 95-99. • Mehta M, Kaur N, Bhutani K: Determination of marker constituents from</p>	
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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 by Affiliated to Manonmaniam Sundaranar University In partial fulfillment of the requirement for the degree of BACHELOR OF SCIENCE IN CHEMISTRY Submitted By M.H.AMEERA 08CH10 B. SELVI Under the Guidance of Dr. A. SYED MOHAMED DEPARTMENT OF CHEMISTRY SADAKATHULLAH APPA COLLEGE (Autonomous) TIRUNELVELI – 11 MAY 2020 DEPARTMENT OF CHEMISTRY CERTIFICATE Certified that the Project work entitled "Antimicrobial activities using Cissus quadrangularis plant root extract" is a work bonafide done by the candidate 17ACH30 has been submitted to Sadakathullah Appa College (Autonomous) for the academic year 2019– 2020. Signature of the Guide Signature of the HOD Place : Tirunelveli Date : Examiner: DECLARATION I hereby declare that the thesis entitle, " Antimicrobial activities using Cissus quadrangularis plant root extract" submitted by me for the Degree of Bachelor in Chemistry is the result of independent research work carried out under the guidance of Dr. A. SYED MOHAMED, Assistant Professor and Head, Research Department of Chemistry, Sadakathullah Appa College (Autonomous) has been submitted for the award of any degree, diploma or associate ship, fellowship of this or any other University or any other Institution. Place:Tirunelveli Date :

ACKNOWLEDGEMENTS I raise my heart in deep gratitude to THE ALMIGHTY for HIS guidance and good health that He has given me to carry out my dissertation with the attentiveness and passion. I offer heartfelt thanks to my guide Dr. A. SYED MOHAMED, Assistant Professor and Head, Research Department of Chemistry, Sadakathullah Appa College (Autonomous), Tirunelveli for his inspiring guidance and thought provoking questions with his excellent execution, encouraging attitude, efforts to bring out the best in me and his availability at all time this present study would not have seen the light of day if I did not acknowledge my sincere thanks to our honorable Secretary ALHAJ. T.E.S.FATHU RABBANI, Sadakathullah Appa College (Autonomous), Tirunelveli for his permission to do this project work. I wish to express my gratitude to our principal Dr. M. MOHAMED SATHIK, Sadakathullah Appa College (Autonomous), Tirunelveli for granting permission to do the project skillfully. I wish thanks to Dr. S. Mahadevan (Dean of Science), Dr. S.H. Mohamed Ameen (Controller of Examination), Dr. M. Sheik Muhideen Badhusha, Dr. J. Antony Danish, Dr. P. Jeslin Kanaga Inba , Dr. M. Thameem Ansari, Dr. M.A. Feroz Khan, Dr. S.M.Y Mohamed Muktar Ali and Dr. S. Brillians Revin For their support and help. I extended my thanks to our technical assistant Mr.Feroz Khan for helping me in the Laboratory. I wish my thank to the teaching and non-teaching of Department of Chemistry, Sadakathullah Appa College (Autonomous), Tirunelveli. I remain thankful to all my family members and my friends for their kind enduring support that they offered me doing my thesis work.

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 Antimicrobial method) 4.2.1 Taxonomical classification of *Escherichia coli* 4.2.2 Taxonomical classification of *Shigella flexneri* 4.2.3 Taxonomical classification of *Pseudomonas aeruginosa* 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

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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 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 conce 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 in *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:

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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.

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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

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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

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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 , Ghonusk Harajora Sanskrit Asthibhanga , Asthisamhara, Chaturdharin Chitrankandali, Kandalata, Vajravalli Bengali Harajora Telugu Gudameti ge , Nalleruvajravalli Gujarati Asthisrnkhala (or) Hadasankal Tamil வச்சுரவல்லி

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

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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%

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W

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

Anti- Bacterial studies using *Cissus quadrangularis* Page 12

84%

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

The drug prepared by using this plant

100%

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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 phytochemicals by using various tests. The results 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 compounds: sitosterol (Rao et al., 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. It is a 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 negative bacteria: *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*. 4.2 Antimicrobial activity test (Disk diffusion method): The disk diffusion test, or agar diffusion test, or Kirby-Bauer test, or 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 antibiotics. The discs 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 bacteria, there will be a zone of inhibition around the disc.

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 inhibition. The size of the zone of inhibition is measured. *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* were prepared. 2. Nutrient agar plates were prepared and kept for drying. 3. Pathogens were taken in a sterile swab. 4. The pathogens 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, control were mentioned on the plates. 7. Incubation at 37 °C. 8. After 24 hours the plates were taken and zone of inhibition were measured using ruler. Bacteria used: ? *Escherichia coli* ? *Pseudomonas aeruginosa* ? *Shigella flexneri* ? *Staphylococcus aureus* ? *Pseudomonas fluorescens*

Anti- Bacterial studies using *Cissus quadrangularis* Page 17 4.2.1 Taxonomical classification of *Escherichia coli* Kingdom Bacteria Phylum Proteobacteria Class Gammaproteobacteria Order Enterobacteriales Family Enterobacteriaceae Genus *Escherichia* Characteristic features of *Escherichia coli*: *Escherichia coli* (commonly abbreviated E.coli) is a Gram-negative, facultatively anaerobic, rod shaped bacterium that inhabits 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 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 other facultative constitute about 0.3% of the normal flora. Transmission is the major route through which pathogenic strains of the bacterium cause disease. Cells are able to survive outside the body for several hours.

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 has been a lot 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 *Shigella flexneri* Kingdom Bacteria Domain Bacteria Phylum Proteobacteria Class Gammaproteobacteria Order Enterobacteriales Family Enterobacteriaceae Genus *Shigella* Species *S. flexneri* Characteristics features of *S. flexneri* *Shigella flexneri* is a species of 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 mammalian large intestine. The 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 aeruginosa*: Kingdom Bacteria Phylum Proteobacteria Class Gammaproteobacteria Order Gammaproteobacteria Family Pseudomonadaceae Genus *Pseudomonas* Characteristics features of *Pseudomonas aeruginosa* *Pseudomonas aeruginosa* is a common Gram-negative rod-shaped bacterium that can cause a variety of infections in animals, including humans. A species of considerable medical importance, *P.aeruginosa* is a prototypical "multidrug resistance (MRD) pathogen" that is recognized for its ubiquity, its intrinsic ability to form biofilms, and its association with serious illness, especially nosocomial infections such as ventilator associated pneumonia and various sepsis syndromes. The organism is considered opportunistic because infection is often superimposed upon acute or chronic morbidity, most notably cystic fibrosis and traumatic burns – or found in immuno-compromised individuals, but the organism is also a common cause of infection in healthy individuals.

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 immunity is present. Tuberculosis, meningitis, and folliculitis. 4.2.4 Taxonomical classification of *Staphylococcus aureus*: Kingdom Bacteria Phylum Firmicutes Class Bacilli Order Bacillales Family Staphylococcaceae Genus *Staphylococcus* Characteristic features of *Staphylococcus aureus* 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 subspecies are recognized: *S. aureus* and *S. aureus* subsp. *aureus*. There are 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 grow on a wide variety of media.

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 diseases in humans, 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 staphylococcal infections are bacterial infections. Antibiotics is basically used. They are commonly cephalosporins, nafcillin, sulfadiazine, vancomycin. Vancomycin is highly used. 4.2.5 Taxonomical classification of *Pseudomonas fluorescens*: Kingdom Bacteria Domain Bacteria Phylum Proteobacteria Class Gammaproteobacteria Order Pseudomonadales Family Pseudomonadaceae Genus *Pseudomonas* Species group *Pseudomonas* Species: *P. fluorescens* *Pseudomonas fluorescens* is a common Gram-negative, rod-shaped bacterium. It belongs to the *Pseudomonas* genus. It has an extremely diverse metabolism, is highly motile, and is a common soil bacterium. 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 tests positive for catalase, oxidase, and nitrate reductase. It 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 medium

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 ground in a mortar and pestle 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 hrs. The mixture was filtered through 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* species were carried out 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) and 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 - Burchard's Test: 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

50%

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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_2O 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 in a water bath. Formation of 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 more ml of Mayer's reagent 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 Dragendorff's added. Formation of orange - brown precipitate indicates the presence of 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 portion of test solution, 2ml of Hager's reagent is added. Formation of white precipitate shows the 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 the presence of phenolic compounds. e) Test for saponins: A portion of the test solution is shaken well with 5ml of water. Formation of 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 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. ii) Ferric chloride Test: A small portion of the test solution is treated with 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. Formation of pink colour 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

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 much or too little cotton. 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 pipette gently to settle 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 about 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 Column

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 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 silica gel into the pipette. The 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 about 10 ml of the eluting solvent with 10 ml of 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 the column. Under these 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 ether. Collect the 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. After the solvent has evaporated, weigh the beakers at 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 its exactly value.

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 1. 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 of *Cissus quadrangularis*. 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 Positive 8. 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 fluorescens* and *Pseudomonas aeruginosa* with 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 that the 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 *Cissus quadrangularis* was found to be remarkable. The extracts revealed remarkable inhibitory activity against gram positive and gram negative bacterial pathogens. Treatment of diseases possesses challenging problems due to emerging infectious diseases and a large 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 plant 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* 64: 49-53. 2. 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, August 1997, Pages 385-390. 3. 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 chemical constituents of *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 *Peltandra* 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 properties, may be of immense importance in therapeutic treatments.
W [https://ijpsr.com/bft-article/phytochemical-screening-and-antibacterial-activity-of-gymnema-sylve ...](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 Clade: Rosids Order: Vitales Family: Vitaceae Genus: *Cissus* Species: *C. quadrangularis* Binomial name quadrangularis
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 plant is a fleshy, cactus-like climber widely used as a common food item in India. This plant
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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, flatulence, skin diseases, leprosy, hemorrhage, epilepsy, convulsion, haemoptysis, tumors, chronic ulcers, swellings.
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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 stem possess antibacterial activity against *S. aureus*, *E. coli*, and *P. aeruginosa* and mutagenicity against *Salmonella* microsome 13 .
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7/26	SUBMITTED TEXT	23 WORDS	94% MATCHING TEXT
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8/26	SUBMITTED TEXT	19 WORDS	MATCHING TEXT
<p>prevent gastric damage 16 . Analgesic, anti-inflammatory and stimulatory activity: Methanol extract</p> <p>W https://www.ijppsjournal.com/Vol3Suppl4/2439.pdf</p>		<p>prevent gastric damage. Analgesic, anti-inflammatory and stimulatory activity [31]</p>	
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<p>analgesic, anti-inflammatory and venotonic effects associated with hemorrhoids, anti-inflammatory 17 .</p> <p>W https://www.ijppsjournal.com/Vol3Suppl4/2439.pdf</p>		<p>analgesic, anti-inflammatory and venotonic effects associated with hemorrhoids, anti-inflammatory 17 .</p>	
10/26	SUBMITTED TEXT	15 WORDS	84% MATCHING TEXT
<p>Toxicology: Cissus quadrangularis extract does not show any toxic effect on oral administration.</p> <p>W https://www.ijppsjournal.com/Vol3Suppl4/2439.pdf</p>		<p>Toxicology [45] The Cissus quadrangularis extract does not produce any toxic effect on oral administration.</p>	
11/26	SUBMITTED TEXT	13 WORDS	100% MATCHING TEXT
<p>is safe even at higher dose for a prolonged duration of treatment</p> <p>W https://www.ijppsjournal.com/Vol3Suppl4/2439.pdf</p>		<p>is safe even at higher dose for a prolonged duration of treatment.</p>	
12/26	SUBMITTED TEXT	48 WORDS	95% MATCHING TEXT
<p>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 .</p> <p>W https://www.ijppsjournal.com/Vol3Suppl4/2439.pdf</p>		<p>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. [20]</p>	
13/26	SUBMITTED TEXT	22 WORDS	50% MATCHING TEXT
<p>and 2ml of concentrated sulphuric acid is added along the sides of the test tube. Formation of purple colouration at the</p> <p>W https://www.doc-developpement-durable.org/file/Arbres-Bois-de-Rapport-Reforestation/FICHES_ARBRES ...</p>		<p>and 2ml of H₂SO₄ was added along the sides of the test tube and the formation of purple colouration at the</p>	
14/26	SUBMITTED TEXT	16 WORDS	66% MATCHING TEXT
<p>with equal volumes of Tollens reagents A and B and heated in a water bath.</p> <p>W https://www.doc-developpement-durable.org/file/Arbres-Bois-de-Rapport-Reforestation/FICHES_ARBRES ...</p>		<p>with equal volumes of Fehling's solution A and B and heated in boiling water bath.</p>	
15/26	SUBMITTED TEXT	17 WORDS	82% MATCHING TEXT
<p>with equal volumes of Fehling's solution A and B and then heated in a water bath.</p> <p>W https://www.doc-developpement-durable.org/file/Arbres-Bois-de-Rapport-Reforestation/FICHES_ARBRES ...</p>		<p>with equal volumes of Fehling's solution A and B and heated in boiling water bath.</p>	
16/26	SUBMITTED TEXT	31 WORDS	84% MATCHING TEXT
<p>Jaiswal S, Singh S V, Singh B, Singh HN. Plants used for tissue healing of animals. Natural Products Radiance. 2004; 3: 284-92.</p> <p>W https://www.ijppsjournal.com/Vol3Suppl4/2439.pdf</p>		<p>Jaiswal S, Singh SV, Singh B, Singh HN. Plants used for tissue healing of animals. Natural Products Radiance. 2004; 3: 284-92. 39.</p>	

17/26	SUBMITTED TEXT	41 WORDS	100% MATCHING TEXT
Luseba D, Elgorashi EE, Ntloedibe DT, Staden JV. Antibacterial, anti-inflammatory and mutagenic effects of some medicinal plants used in South Africa for the treatment of wounds and retained placenta in livestock. South African Journal of Botany. 2007; 73: 378-83. 8.		Luseba D, Elgorashi EE, Ntloedibe DT, Staden JV. Antibacterial, anti-inflammatory some medicinal plants used in South Africa for the treatment of wounds and retained placenta in livestock. South African Journal of Botany 2007; 73: 378-83. 30.	
W https://www.ijpsjournal.com/Voi3Suppl4/2439.pdf			
18/26	SUBMITTED TEXT	27 WORDS	100% MATCHING TEXT
These findings clearly demonstrated that the bioactive metabolites present in <i>Cissus quadrangularis</i> can be used for the treatment of disease.		These findings clearly demonstrated that the bioactive metabolites present in <i>C</i> used for the treatment of disease. •	
W https://www.journalijar.com/article/17095/antimicrobial-activities-of-silver-nanoparticles-synthe...			
19/26	SUBMITTED TEXT	18 WORDS	82% MATCHING TEXT
Adesanya SA, Nia R, Martin MT, Boukamcha N, Montagnac A, Pais M. Stilbene derivatives from <i>Cissus quadrangularis</i> .		Adesanya SA, Nia R, Martin M, Boukamcha N, Montagnac A, Pas M. Stilbene deri quadrangularis.	
W https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5115982/			
20/26	SUBMITTED TEXT	12 WORDS	100% MATCHING TEXT
Studies on " <i>Cissus Quadrangularis</i> " in fracture by using phosphorus 32. III.		Studies on " <i>Cissus Quadrangularis</i> " in fracture by using phosphorus 32. III	
W https://www.researchgate.net/publication/285707665_Pharmacological_and_therapeutic_activity_of_Ci...			
21/26	SUBMITTED TEXT	12 WORDS	100% MATCHING TEXT
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...			
22/26	SUBMITTED TEXT	40 WORDS	64% MATCHING TEXT
of <i>Cissus quadrangularis</i> . Afri J of Biomed Res. 2005. 8, 95-99. 12. Mehta M, Kaur N, Bhutani K., Determination of marker constituents from <i>Cissus quadrangularis</i> Linn and their quantitation by HPTLC and HPLC. Phytochem Anal, 2001. 12. 91-105. 13.		of <i>Cissus quadrangularis</i> Int J Green Mehta M, Kaur N, Bhutani KK. 2001. Detern constituents from <i>Cissus quadrangularis</i> Linn. and their quan- titation by HPTLC 12:91-95.	
W https://www.researchgate.net/publication/230849528_A_Review_and_Evaluation_of_the_Efficacy_and_Sa...			
23/26	SUBMITTED TEXT	18 WORDS	95% MATCHING TEXT
T, Saifah E., Constituents of <i>Cissus quadrangularis</i> Linn. J Pharm Sci., 1986, 11, 205- 11.		T, Saifah E. Constituents of <i>Cissus quadrangularis</i> Linn Thai J Pharm Sci 1986;11	
W https://greenpharmacy.info/index.php/ijgp/article/view/255/266			
24/26	SUBMITTED TEXT	26 WORDS	91% MATCHING TEXT
Prasad G.C., Udupa K.N., Effect of <i>Cissus quadrangularis</i> on the healing of cortisone treated fracture, Indian Journal of Medical Research, 1963, 51, 667. 16. Prasad		PRASAD, G. C., & UDUPA, K. N. (1963). EFFECT OF CISSUS QUADRANGULARIS C CORTISONE TREATED FRACTURES. The Journal of Medical Research, 51, 667-7	
W https://www.unboundmedicine.com/medline/citation/14073618/EFFECT_OF_CISSUS_QUADRANGULARIS_ON_THE_...			
25/26	SUBMITTED TEXT	18 WORDS	100% MATCHING TEXT
of a phytogetic steroid from <i>Cissus quadrangularis</i> , Journal of Research in Indian Medicine, 1972, 4, 132. 17.		of a phytogetic steroid from <i>Cissus quadrangularis</i> . Journal of Research in India	
W https://pharmacologyonline.silae.it/files/archives/2007/vol3/15_Rao.pdf			
26/26	SUBMITTED TEXT	19 WORDS	83% MATCHING TEXT
Udupa K.N., Prasad G.C., Sen S.P., The effect of phytogetic steroid in the accleration of fracture repair, Life		Udupa, K.N., Prasad, G. and Sen, S.P., 1965. The effect of phytogetic anabolic str fracture repair. Life	
W https://escipub.com/ajcrr-2020-07-1705/			

