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Bioactive Compounds, Pharmacological Activities and Its Applications from Adamant Creeper/Veldt Grape

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ABSTRACT: *Cissusquadrangularis* belongs to a taxonomical group of Magnoliopsida and a family of Vitaceae. *Cissusquadrangularis* is an ancient medicinal plant native to India and Sri Lanka. It has been used in India for its healing process. It's commonly known as a "hadjora" in Hindi and "pirandai" in Tamil. It was described in the ancient Ayurveda texts as a general tonic which containing specific healing properties. It is asserted that it can be utilized for the remedy of diabetic, cancer and can be further used as an antimicrobial activity and antioxidant activity.

KEY WORDS: Anti-cancer, Anti-diabetic, Antioxidant, Anti-microbial activity,*Cissusquadrangularis.*

I. INTRODUCTION

Medicinal plants have been used as a traditional treatment for numerous human diseases from ancient times all around the world. More than 40% of the entire plant species was used for medicinal purposes. Herbal products today symbolize safety in contrast to other synthetic products which resembles unsafe to humans and the environment. Medicinal plants play a vital role in the management of diseases in developing countries where resources are exiguous. Herbal medicine is based on the preface that plants contain natural substances which can promote human health and mitigate illness. The most important of these biologically active compounds of plants are flavonoids, tannins, alkaloids, and phenolic compounds. *Cissusquadrangularis* brought the attention of worldwide researchers for its pharmacological activities such as anti-microbial, anti-cancer, anti-diabetic, and antioxidant. The objective of this article is to review the botanical description, bioactive compounds, anti-cancer, anti-diabetic, antioxidant activity, and applications.

Cissusquadrangularis a vine that belongs to the family of Vitaceae. The most oftentimes used medicinal plants in India. It is an edible plant found in the warmer regions of India, Sri Lanka, and West Africa. It is mainly native to India or Sri Lanka but also found in Africa and Southeast Asia.

Cissusquadrangularis is a perennial plant of the grape family. It is commonly called as veldt grape or devil's backbone. It is referred to as "Asthisandhani" in Sanskrit. The fresh stem and leaves of Cissusquadrangularisare used for the treatment of a menstrual disorder, scurvy and flatulence.

PLANT CLASSIFICATION

Kingdom: Plantae; Division: Magnoliophyta; Class: Magnoliopsida; Order: Vitales; Family: Vitaceae; Genus: Cissus; Species: quadrangularis.

Fig 1: Photograph of *Cissusquadrangularis*.

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II. BOTANICAL DESCRIPTION

Cissusquadrangularis is a plant that has been esteemed for its medicinal properties for thousands of years. Anciently, it was utilized to treat many diseases like gout, asthma, and allergies. *Cissusquadrangularis* attains a height of 1.5 m (4.9 ft.) and has quadrangular-sectioned branches with internodes of 8-10 cm (3-4 in) long and 1.2 -1.5 cm (0.5-0.6 in) wide. And each angle is a leathery ledge. Toothed trilobed leaves are about 2-5 cm (0.8-2.0 in) wide. Each has a tendril originating from the opposite side of the node. Racemes of small white, yellowish, or greenish flowers; globular berries are red wine ripe.
Cissusquadrangularis is an evergreen climber. Its hardy zone is suitable for light (sandy), medium (loamy), and heavy (clay) soils, prefers well-drained soil, and can grow in nutritionally poor soil.
Suitable pH: acid, neutral, and basic soils and can conveniently grow in very acidic and very alkaline soils. It cannot grow in the shade. It prefers dry or moist soil and can also tolerate drought. *Cissusquadrangularis* contains carotenoids, triterpenoids, and ascorbic acid. The plant also produces the resveratrol dimer quadrangularin.

III. PHARMACOLOGICAL ACTIVITIES

N.D Kashikar, and Indu George(2006) Antibacterial activity of*CissusQuadrangularis Linn.* Theyexamined in-vitro antibacterial activity from *Cissusquadrangularis Linn* against Gram-negative and Gram-positive bacteria,where the methanol and ethyl acetate extract showed high activity against the bacteria. With the help of the minimum inhibitory concentration (MIC), the values of the different extracts of the plant stem are recorded using the microtitre plate method.The samples were washed, cleaned, and cut into small sections, and dried in the oven. The dried material is grind and stored in the refrigerator. Extraction was carried out by using the Soxhlet apparatus.
*Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa***,** and *Staphylococcus aureus* were obtained. Bioassays were performed in 96-well sterile microtitre plates. The MIC values of different extracts against all the organisms were tested, and are detailed in Table. The ethyl acetate, acetone, and methanol extracts showed antimicrobial properties in*B. subtilis, P. aeruginosa,S. Typhi, S. aureus,* and *S.pyogenes* were liable to at least two extracts. Petroleum ether, ethanol, and water extracts were failed to inhibit the bacterial growth of the strains tested**.** *E.coli* did not respond to any of the extracts used.

Table 1: MIC values of test organisms used against various extracts (MIC values in mg/ml).

Extract	*E. coli*	*B. subtilis*	*P. aeruginosa*	*S. typhi*	*S. aureus*	*S.pyogenes*
Petroleum ether	-	-	-	-	-	-
Ethyl acetate	-	0.93	1.87	3.75	0.93	3.75
Acetone	-	-	3.125	6.25	1.56	-
Methanol	-	0.465	3.12	1.24	0.465	0.93
Ethanol	-	-	-	-	-	-
Water	-	-	-	-	-	-

All values are expressed as the mean of three experiments. '-' indicates no inhibition.Moreover, CD analysis was carried out to pinpoint the best extract and the most liable microorganism.
K.N. Chidambara Murthy(2003) - Antioxidant and antimicrobial activity of*Cissusquadrangularis L.*This paper examined antioxidant activity by expending β- carotene linoleic acid model and 1, 1-diphenyl-2-picrylhydrazyl model. The ethyl acetate fraction of both samples were extracted which showed 64.8% antioxidant activity in β- carotene linoleic acid, and 61.6% in 1, 1-diphenyl-2-picrylhydrazyl system. Fraction showed the presence of sterols, vitamin C, and tannins as phytoconstituents.
Antioxidant activity of methanol extract and aqueous extracts were proximately less vital than that of ethyl acetate extract, and n-hexane extract indicated the limited activity. Ethyl acetate extract and methanol extract of both fresh and dry stems were further demonstrated for antimicrobial activity against Gram-positive bacteria, which includes *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus*, and *Streptococcus species.*

Solvents and chemicals are used in this experiment are:-
I. Analytical or high-performance liquid chromatography (HPLC).
II. Ultraviolet and visible spectra measurements.
For antimicrobial assays, six bacterial cultures were selected:

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a. *Bacillus subtilis,*
b. *Bacillus cereus,*
c. *Staphylococcus aureus,*
d. *Pseudomonas aeruginosa,*
e. *Escherichia coli,*
f. And *Streptococcus species*.

In vitro antibacterial activity was determined by obtaining nutrient broth agar.
Cissusquadrangularis were collected and authenticated. Cleaned stems are divided into two batches, the first batch was directly subjected to extraction using Soxhlet extractor and the other batch was dried under vacuum to dry stems. The powdered sample is then subjected for extraction. N-hexane, ethyl acetate, methanol, and water are used for extraction.The dry mass is added and made up of 1% solution in the respective solvents and subjected for phytochemical screening (sterols, tannins, alkaloids, carbohydrates, and ascorbic acid.)
DPPH radical scanning assay: Different concentrations of C.Q are extracted using butylatedhydroxyanisole. The control was prepared, and methanol was used as a baseline correction in OD. Radical scavenging activity is expressed as % scavenging activity and is calculated by the following formula:

%Radical scavenging activity= (Control OD- Sample OD/ Control OD) ×100

B-CLAMS antioxidant assay: The antioxidant activity of C.Q extract was assessed by the B-CLAMS. The resulting mixture was diluted in triple-distilled water and mixed well. The emulsion is prepared with oxygenated water. BHA is used for comparative purposes. The antioxidant activity (AA) of the extracts was calculated in terms of bleaching of the β-carotene using the following formula:

$$AA = 100[1- (A_0 – A_t)/ (A^0_0- A^0_t)]$$

Where A_0 and A^0_0 are the absorbance values measured at zero time of the incubation for test sample and control, and $A_t$and A^0_t are the absorbance values measured in the test sample and control after incubation for 180 minutes.
Anithaet.al (2010) Antimicrobial profile of*CissusQuadrangularis.*This articleexamined antimicrobial activities opposed *E.coli, AspergillusNiger, Pseudomonas aeruginosa*,*Penicilliumsp, Mucorsp, Candida albicans* by employing disc diffusion method. Five solvents were depleted for extraction and they are, Petroleum ether, Chloroform, Ethyl acetate, Ethanol and aqueous extracts. Among those five extracts Aq. Extract showed utmost antibacterial and antifungal activity against *Pseudomonas aeruginosa*and *Mucor sp*. Phytochemical screening admitted the presence of alkaloids, flavonoids, Saponin, steroids, tannins, amino acids and proteins. Fresh plant of *Cissusquadrangularis* was used. The plant was dried and milled into a fine powder and extracted with petroleum ether, ethyl acetate, chloroform, ethanol and aq. Extract. The extract was filtered through Whitman paper and evaporated for dryness under reduced pressure using rotary evaporator. Phytochemical screening was done to identify the secondary metabolites in plant.
Bauer's et al. antimicrobial activity method was acknowledged with slight modification where Nutrient agar medium was used in sterile Petri plates for test culture and sterile discs were dipped in solution of different conc. of various extracts and dried. The dipped disc was used as a negative control and standard antibacterial agent chloramphenicol disc was used as a positive control. The plates were incubated and measured for antibacterial activity. The diameter of the inhibition zone was measured in mm and the antibacterial were performed in triplicates and values were expressed in Mean ±Standard derivation.
Antifungal activity was assayed using the same procedure of antibacterial activity in Sabouraud Dextrose Agar (SDA) medium. The standard streptomycin was used as positive control, and plates were incubated. The diameter of the inhibition zone was measured in mm and the antifungal experiments were performed in triplicates to obtain the data.

ANTI-CANCER ACTIVITY

P Sureshet.al(2019),Anti-cancer activity of *cissusquadrangularis l.* methanolic extract against mg63 human osteosarcoma cells- and in-vitro evaluation using cytotoxicity assay. In this article they investigated that alcoholic extract of plant displayed anti-cancer activity against all cell lines derived from cervical, skin, colon, breast, and kidney cancers. The flavonoid fraction of the extract was found to be the active compound for the activity. Methanolic extract of C.Q was prepared and its anticancer activity was tested in cell lines using the Mossman method of cytotoxicity assay. C.Q plants were collected and authenticated. The aerial parts of the plants were dried and powdered, and was subjected to Methanolic extraction using the soxhlet apparatus. Dulbecco's Modified Eagle Media (DMEM) was used for maintaining cell line, which was augmented with Fetal Bovine Serum (FBS). Penicillin and streptomycin were put into the medium to avert bacterial contamination. Medium with cell lines was retained in a humidified environment. Cells are placed in 24-well plates and incubated with 5% of CO_2 condition. Cells are placed in wells that reached confluence

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and the prepared concentrations of extract were added and kept in an incubator. Samples were removed from the wells and washed with phosphate-buffer saline or DMED without serum. 0.5% of 3- (4, 5- dimethyl-2- thiazolyl)-2, 5-diphenyl- tetrazolium bromide (MTT) was added to each well and incubated for 24 hours. DMSO was added to all wells to dissolve the formazan crystals. Each sample was placed in the cuvette, DMSO used as a blank to measure the absorbance value and the wavelength using an ultraviolet (UV) spectrophotometer. From three observations the average absorbance values are observed. Observed values are tabulated, and the concentration required for the inhibition is determined graphically. Percentage cell viability is calculated by determining the ratio between A570 of treated cells, A570 of control cells is multiplied by 100. Cell control and sample control are involved in each assay to differentiate the cell viability assessments.The cell viability and the cytological characteristics of MG63 cells are observed in the microscopic images of cells treated with the extract.

Table 2: Cell viability of MG63 cells treated with *Cissusquadrangularis* Methanolic extract.

S.No	Extract concentration (µg/ml)	Dilution	Absorbance at 570 nm	% Cell viability
1	1000	Neat	0.602	29.65
2	500	1:1	0.721	35.51
3	250	1:2	0.828	40.78
4	125	1:4	0.989	48.71
5	62.5	1:8	1.112	54.72
6	31.2	1:16	1.245	61.33
7	15.6	1:32	1.344	66.20
8	7.8	1:64	1.494	73.59
9	Cell control	–	2.030	100

O.D – Optical Density, % – percentage, Percentage cell viability at graded concentrations of plant extract.

ANTI-DIABETIC ACTIVITY

Srivastava et.al (2011) Anti-diabetic activity of the stem extracts of*Cissusquadrangularis L*. Here they examined that stem of C.Q was used for an anthelmintic, laxative, chronic ulcer, and bone healing activity. The whole plant is used for bitter, sweet, sour, fracture swelling, and also used in Diabetes. Flavonoids and triterpenoids were the active constituents of the stem of C.Q and responsible for its pharmacological activities. The study shows some useful diagnostic inventories for the identification and preparation of monographs of the plant. In this paper ethyl acetate and hydro alcoholic extract indicated crucial anti-diabetic activity. The stem of the plant was collected and authenticated. The stem is transversely cut, dried, powderedand stored. The dried powder was extracted with ethyl acetate through cold maceration. The heterogeneous powder was stirred four times a day. Extraction was continued for 72 hours. The ethyl acetate extract is filtered and concentrated in a syrupy mass using vacuum distillation. Brown residue is obtained. The dried residue was macerated with alcohol for 72 hours. The hydro alcoholic extract was obtained, filtered, and concentrated using vacuum distillation. Blackish brown residue is obtained. The extract and powder were subjected to various chemical tests to observe the presence of specific phyto-constituents.

Selected diabetic animals were erratically split-up into four groups. One group is assisted as a control, the second group obtained the standard drug Glibenclamide and the other groups are regenerated in the suspension of Gum acacia. Anti-diabetic activity of C.Q was investigated using an alloxan-induced diabetes model. Alloxan-induced diabetes model was specified to verify the efficacy of the active anti-hyperglycemic extract in practical diabetic conditions. After 72 hr of injection, the fasting blood glucose level was measured. Animals that did not develop more than 200 mg/dl glucose levels were rejected. Statistical analysis was done by obtaining ANOVA which reveals the valid difference between the test group and the control group. And the data were expressed as Mean ± SEM; $P<0.05$ implicates significance.

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ANTIOXIDANT ACTIVITY

Vijayalakshmi et.al (2013)in vitro antioxidant and anticancer activity of flavonoid fraction from the aerial parts of *cissusquadrangularis l.* against human breast carcinoma cell lines. Theyexamined that flavonoid extraction was isolated from the aerial part of C.Q to evaluate its antioxidant activity and anticancer potential using in vitro assay system. Total phenolic and flavonoid volume were evaluated for the drug. Column chromatography was used for flavonoid fraction, isolated, and analyzed using HPLC. In vitro, antioxidant activity and isolated flavonoid fraction were examined by nitric acid, DPPH, and hydroxyl radical scavenging assay. A breast cancer cell line was used in the cancer model for the MTT assay. The total phenolic content and total flavonoid content in ethanol extract amount are showed using dry weight expressed as Gallic acid equivalents. The tested extract showed quantity free radical scavenging property in entire models for ethanol extract and flavonoid fraction. Flavonoid fraction acquires anticancer property against breast cancer cells. The plant extract was prepared from powdered aerial C.Q and defatted by petroleum ether in soxhlet apparatus. The defatted powder was macerated by ethyl alcohol to obtain the alcoholic extract. Concentrated extraction was obtained using the rotavapore apparatus. Phytochemical screening was done by using Conc. Ethanol in standard procedure. TLC was performed using ethanol extract, and satisfactory result was obtained in the mobile phase Benzene: Methanol: Ammonia (9:1:0.1) and silica gel F as stationary phase. The plate was dried and accessible to ammonia vapor and specific flavonoid is detected.In vitro antioxidant activity requires various concentrations of ethanol extract and total flavonoid fraction which were tested for their antioxidant activity in different in vitro models. Free radical scavenging property was observed in the above test.

BIOACTIVE COMPOUNDS

KoudoroYaya Alainet.al(2015) Chemical characterization and biological activities of extracts from two plants (*Cissusquadrangularis*, and*Acacia polyacantha*) USED IN VETERINARY MEDICINE IN BENI. This study claimed the presence of bioactive compounds in his article. The plant used was *Cissusquadrangularis* and *Acacia polyacantha.* Trunk bark of C.Q and *A. polyacantha* were used as a plant material. The plant material was dried and powdered in a laboratory condition.

Analyzing of Bioactive compounds-

The dried powdered samples were used for the further tests and they are;

- **Flavonoid**- Presence of flavonoid was carried out by cyaniding test.
- **Alkaloid**- Presence of alkaloid was carried out by Meyer test and it is confirmed by Bouchardat test.
- **Tannin-** The reaction was carried and highlighted by Stiasny test.
- **Saponin-** Presence of Saponin was identified by foam test.
- **Polyphenol-** Ferric chloride test was done for the conformation of polyphenol compound.
- **Terpens and Sterols-** Liebermann-Burchard tests are the conformation tests for terpens and sterols.
- **Anthraquinone-** Presence of Anthraquinone was done by Borntrager test.

In plant *A. polyacantha* Alkaloid, Tannin, Saponin, Sterol and Terpens showed positive result whereas, Polyphenol, Flavonoid and Anthraquinone showed no reaction.And in plant *C. quadrangularis* Tannin, Polyphenol, Flavonoid, Sterol and Terpens showed positive result while Alkaloid, Saponin, and Anthraquinone showed negative result.

Table 3: Analyzing bioactive compounds using *Cissusquadrangularis*.

Secondary metabolites	***A. polyacantha***	***C. quadrangularis***
Alkaloid	+	-
Tannin	+	+
Sterol and Terpens	+	+
Polyphenol	-	+
Flavonoid	-	+
Saponin	+	-
Anthraquinone	-	-

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IV. CONCLUSIONS

Cissusquadrangularis exhibits various pharmacological activities such as antibacterial, antioxidant, anti-diabetic, anticancer and biological active compounds. The ethyl acetate, acetone and methanol showed antimicrobial properties whereas, ethanol, petroleum ether and water extracts failed to show antimicrobial properties in N.D Kashikar and Indu George article. K.N Chidambara et.al sights the antioxidant activity by obtaining β-carotene linoleic acid model and 1, 1-diphenyl-2-picrylhydrazyl model.Anitha et.al verified antimicrobial activity by employing disc diffusion method with the help of five solvent extracts. P Suresh et.al examined alcoholic extract from plant C.Q which displays anti-cancer activity against all cell lines derived from cervical, skin, colon, breast, and kidney cancers. A.K Srivastava et.al investigated that whole plant of C.Q can be used for diabetes. Flavonoids and triterpenoids are the active constituents of the stem of C.Q, and ethyl acetate and hydro alcoholic extract indicated the crucial anti-diabetic activity.

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