PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITIES OF STEM OF CISSUS QUADRANGULARIS PLANT

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Abstract : *Present study reports phytochemical screening and the microbial activity of crude extracts of Stem of Cissus quadrangularis Plant on some human pathogenic bacteria and fungi.Cissus quadrangularis has been used in the traditional medicines of various cultures, the plant has been mentioned in all ancient scriptures of Ayurveda. It is used in folklore medicine to heal bone fractures, throughout India. Since then it has been in extensive use by bone setters both for external application and as an internal medicine to be taken with milk. The stem of Cissus quadrangularis is also reputed in Ayurveda as alterative, anthelmintic, dyspeptic, digestive, tonic, analgesic in eye and ear diseases, in the treatment of irregular menstruation and asthma, in complaints of the back and spine. Scientific studies have revealed the Cissus extract to possess cardiotonic and androgenic property. The zone of inhibition was determined utilizing the well diffusion method. Ethanol was found to be highly sensitive against Gram positive S-aurous and gram-negative E-coli.The inhibitory activity of these extract confirmed the potential use of the plant in the treatments of microbial induced ailments.*

Keywords : *Phytochemical screening, Cissus quadrangularis, pharmacologically, Antimicrobial activity*

Introdution :

Cissus quadrangularis Linn. (Family: Vitaceae) is commonly distributed thorough out the hotter parts of India and Sri Lanka. In India the plant has several other names in different languages such as in Hindi: Hadjora; Bengali: Harjora; Gujrati: Hadasankala; Oriya: Hadjodi; Punjabi: Hadjjor; Malayalam: Piranta; Tamil: Pirantai; Telgu: Nalleru and English: Edible stemmed vine. In Sanskrit Cissusquadrangularis known as asthisamharaka literally means, that which saves the bones from their destruction. Precisely, it is also named as asthisandhani, which describes its peculiar quality of healing the bone fractures. Denoting the same property, is has few synonyms, like vafravati, asthisrnkhala, vajrangi, granthimala etc.Cissus quadrangularis is a succulent vine from Asia and Africa. It is one of the most commonly used medicinal plants in Thailand. It is traditionally used in African medicine as well as in Ayurveda. All parts of the plant are used for medicine. Cissus quadrangularis is a traditional medicine usually said to come from Ayurveda but appears to have a wide range of locations which have used it medicinally due to its growing in numerous locations. Traditionally it was

mostly used in treatment of female disorders (menopause, libido, and menstrual disorders) and treating bone disorders (increasing bone mass or accelerating fracture healing rates) which gives it the traditional name of the 'Bone Setter' (Hadjod), some other traditional usages are in regards to its supposed antiulcer properties, Antihemorrhoid properties, pain relieving properties and wound healing properties .Cissus quadrangularis is a low-growing shrub with a characteristic, four-sided stem. It is a climbing plant, often found growing over lower growing vegetation. Cissus' thick stem is glabrous and fleshy, with constrictions at its nodes. Its alternate, simple leaves are also thick and ovate, with serrated margins. The leaves measure about 8 cm long and 6 cm broad. Numerous tendrils grow out of the plant's nodes. Pharmacognostical Studies [4] a) Macroscopic Drug occurs as pieces of stem of varying lengths; stern quadrangular, 4-winged, internodes constricted at nodes; a tendril occasionally present at nodes; internodes 4-15 cm long and 1-2 cm thick; surface smooth, glabrous, buff coloured with greenish tinge, angular portion reddish-brown; no taste and odour.

The stout, fleshy quadrangular stem is traditionally used for the treatment of gastritis, bone fractures, skin infections, constipations, eye diseases, piles, anemia, asthma, irregular menstruation, burns and wounds. The leaves and young shoots are powerful alternatives. Powder is administered in treatment of hemorrhoids and certain bowl infections. The juice of stem is useful in scurvy and in irregular menstruation whereas the stem paste boiled in lime water is given in asthma. It is also used as a powerful stomachic. Cissus quadrangularis Linn. has potent fracture healing property and antimicrobial, antiulcer, antioxidative, antiosteoporotic, gastroprotective, cholinergic activity as well as beneficial effects on cardiovascular diseases.

C. quadrangularis treatment has increased the DNA synthesis of human osteoblastic SaOS-2 cells indicating increased proliferation of these cells]. The study also revealed that the anabolic actions of ethanolic extract of C. quadrangularis in human osteoblast like cells are mediated through increased mRNA and protein expression of Runx2, a key transcription factor involved in the regulation of bone matrix protein. Osteogenic potential of C. quadrangularis on the mandibular fracture healing was studied by and the plant helps in reducing pain, swelling, and fracture mobility and accelerate the healing of fracture jaw bones .

Cissus quadrangularis is used for diabetes, obesity, high cholesterol, bone fractures, allergies, cancer, stomach upset, painful menstrual periods, asthma, malaria, wound healing, peptidase disease, weak bones, weak bones (osteoporosis) and as body building supplements as an alternative to anabolic steroids.

Materials and Method:

Plant Collection:

The present work was carried out at Department of Chemistry, J.M.V. Chandrapur, Gondwana University, Gadchiroli. The plant *Cissus Quadrangularis* was collected from Chandrapur forest region. Their botanical identity of plant was determined and authenticated from literature available in Department of Botany, J.M.V. Chandrapur. The Stem of Cissus

Quadrangularis was thoroughly washed with water and dries under shade for about ten days. The dries plant sample was grind well into a fine powder in a mixture grinder. The powder was stored in a air sealed polythene bag at room temperature before extraction.

Preparation of Ethanol extract:

100g of the dried and powdered *Cissus Quadrangularis* were extracted at room temperature with 500 ml absolute Ethanol extract for 72 hr. extraction was done using Soxhlet apparatus briefly 100gm of powder steam was stored in air sealed polyethene bag and extracted with absolute ethanol. The extraction was done until the solvent in the Soxhlet turned colourless. The extract was concentrated by recovering the solvent using the Soxhlet apparatus until the extract became just pourable. It was poured into a beaker and this was then used for the analysis.

Phytochemical Analysis:

The extracts were analyzed for the presence of Alkaloids, Terpenoids, Tannine, Saponin, Flavonoid, Anthraquinone, Reducing Sugar, Glycoside and Cardiac glycoside.

1. Alkaloid: About 0.2g of the extracts was warmed with 2% H_2SO_4 for two minutes. It was filtered and few drop pf Dragencloffs reagent were added. Orange red precipitated indicated the presence of alkaloids.
2. Tannine: Small quantity of extracts was mixed with water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green solution indicates the presence of tannins.
3. Anthraquinones: About 0.5 g of the extracts was boiled with 10% HCl foe few minutes in a water bath. It was filtered and allow to cool. Equal volume of $CHCl_3$ was added to the filtrate. Few drop of 10% NH_3 were added to the mixture and heat. Formation of rose-pink colour indicates the presence of anthraquinones.
4. Glycoside: The extract was hydrolyzed with HCl solution and neutralized with NaOH solution. A few drop pf Fehling's solution A and B were added. Red precipitate indicates the presence of glycoside.
5. Reducing Sugars: The extracts was shaken with distilled water and filtered. The filtrate was boiled with drop of Fehling's solution for minutes. An orange red precipitate indicates presence of reducing sugar.
6. Saponins: About 0.2g of the extract was shaken with 5ml of distilled water and then heated to boil. Frothing (appearance of creamy miss of small bubbles) shows the presence of saponins.
7. Flavonoids: Extracts of about 0.2g was dissolved in diluted NaOH and HCl was added. A yellow solution that turns colorless, indicates the presence of flavonoids. Phlobatannins: The extract (0.5g) was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl solution. Red precipitated show the presence of phlobatannins.
8. Terpenoids (Salkowski test): 0.2g of extracts was mixed with 2ml Chloroform ($CHCl_3$) and concentrated H_2SO_4 (3ml) was carefully added to form a layer. A reddish

brown coloration of the interface was formed to indicate positive results for the presence of terpenoids.

9. Cardiac glycosides: 5 ml of each extracts was treated with 2ml glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated H_2SO_4. A brown ring of the interface indicates a deoxy sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Preparation of extracts:

The microorganism used in the study: Gram-negative E-coli, Gram – positive S-aurous and Nizer fungus Aspergillus were obtained from stock culture in the Department of Microbiology, J.M.V. Chandrapur.

Antimicrobial Activity:

Antimicrobial activity of callus, different solvent extracts and zinc nanoparticles (Stem). The antimicrobial activities of plant Stem, callus and zinc nanoparticles synthesized used Stem aqueous extract were evaluated by agar well diffusion assay, In each 50ml of plant extract was poured. DMSO was used as a negative control whereas ciprofloxacin was used as positive control. The anti-microbial activity of extract was determined by inhibition zone diameter. The zones are measured by high media zones scale. The experiment was repeated twice and the average values were recorded for anti-bacterial activity.

Antimicrobial screening of extracts:

Susceptibility test were carried out. The modified agar well diffusion method [6,7] to test the antimicrobial activity of the extracts. The medium employed was diagnostic sensitivity agar. The culture was prepared in triplicate was incubated at 370^0 C for 24 to 72 hr. 0.2 ml of the broth culture of the test organism was put in the sterile Petri-dish and 18ml of sterile molten diagnostic sensitivity agar, was added. Well were bored into the medium using 0.1ml of the extracts. Streptomycin and Chloramphenicol were used as the standard antimicrobial agents at a concentration of 10 mcg/disk, 30 mcg/disk respectively. The plates were kept in sterilized inoculation chamber for 2hr to facilate diffusion of the antimicrobial agents into the medium. The plates were then incubated at 37^0C for 24 hr. and the diameter of zone of inhibition of microbial growth were measured in the plates in millimeters.

Result:

Phytochemical screening of ethanol, extract of *Cissus Quadrangularis* is shown in Table No.1. The susceptibility of test microorganism to the crude extracts of *Cissus Quadrangularis* is shown in Table No. 2

Phytochemical tests of ethanol extract of Stem of plant Cissus Quadrangularis

Table No.1

Chemical composition	Ethanol Extract
Alkaloid	Present
Tannine	Absent
Anthraquinone	Absent
Glycoside	Absent
Reducing sugar	Absent
Saponine	Present
Flavonoid	Absent
Terpenoid	Present
Cardiac glycoside	Present

Table No.2.Antimicrobial activity of stem in ethanol

1	Gram +ve s-aureus	Gram -ve E-coli
2	13mm	15mm

Discussion:

The qualitative analysis of extracts from ethanol and Stem of *Cissus Quadrangularis*. Showed the presence of photochemical constituents such as:Ethanol extract present in alkaloid, terpenoid, Saponine, Cardiac glycosides Absent Tannine, flavonoids, anthraquinone, reducing sugar and glycoside. This result is summarized in table 1. The above result indicates that, the Stem of plant extract in ethanol solvent investigated are rich in alkaloid, Tannine, Saponine, flavonoid, terpenoid, cardiac glycoside. The Ethanol extract have showed absence of anthraquinone, reducing sugars and glycoside. Extract of Stem were tested against Gram positive S-aurous and Gram-negative E-coli (with zone of inhibition above 13mm for gram +ve and 15mm for gram -ve means highly sensitive). Ethanol extract was showed more antimicrobial activity than standard antibiotics and chloramphenicol. The The plant studied here can be seen as a potential source of drugs.

Conclusion:

The plant *Cissus Quadrangularis* is rich in alkaloid, Saponine, etc. The antimicrobial activity of the extracts can be co-related to their specific contents loke alkaloids, flavonoids and terpenoids. The ethanol extract was found to be antimicrobials more effective than the hexane extract. The antimicrobial activity of S-aurous is more effective than E-coli. Cissus Quadrangularis and its compound as well as, investigate if these natural products could modulate. The plant summarizes information concerning the morphology, ecology and most importantly phytochemical constituents and antimicrobial activity. Cissus Quadrangularis herb is widely used Tropical countries including India. It has significant traditional uses, some of them have been experimentally established and an attempt has been made to isolate potential chemical constituents and their mechanism of action.

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