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ETHNOMEDICINAL IMPORTANCE OF RARE FAMILY MEMBER OF VITACEAE, *Cissus elongata* Roxb.

Poornima R. Kunder and Vidya SM
Department of Biotechnology
NMAMIT, Nitte, Karkala, Udupi District

***Abstract*– Western Ghats of India is situated along the coastal belt of Arabian Sea is considered to be a global hotspot of biodiversity. Western Ghats contain wide range of flora and fauna. It possesses flora starting from Poaceae *Cynadon dactylon* (L.) Pers. to Dipterocarpaceae *Dipterocarpus indicus* Bedd. *Cissus elongata* Roxb. Sub-species: *littoralis (Talbot) B.V. Shetty & P. Singh* is one such flora belongs to the family. Vitaceae among the wide variety of indigenous medicinal plant found in this region. Ethnic people believed that this plant has been used in the treatment of all types of diseases such as skin and nerve diseases and different types of disorders. In order to know the medicinal properties of this plant ethanol, petroleum ether and methanol: chloroform (3:1) extracts were tested for antioxidant activity by 1-1 Diphenyl 2 Picrylhydrazyl method. Qualitative and phytochemical screening of extracts (secondary metabolites) are tested which showed positive results for flavonoids, alkaloids, terpenoids and tannins. The extracts showed potent antioxidant activity due to the presence of different types of secondary metabolites which may further help in proving their ethno-medicinal applications. The extracts also showed good antibacterial and antifungal activity which implicates the application of *C. elongata* as antimicrobial agent.**

INTRODUCTION

Western Ghats, considered to be the global hotspots of biodiversity, supports an enormous vegetal wealth, covering the states of Goa, Maharashtra, Karnataka, Tamil Nadu and Kerala. These regions encompass a considerable gradient of climatic conditions which have resulted in the development of diverse forest types. These regions house many medicinal plants. Medicinal plants have been used for centuries as remedies for human diseases and offer a new source of biologically active chemical compound. Medicinal plants are the richest bio-resources of drugs of traditional medicinal systems. It has been estimated that 14 - 28% of higher plant species are used medicinally and that 74% of pharmacologically active plant derived components were discovered after following up on ethno medicinal use of the plants. Recently the acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led authors to investigate the antimicrobial and antioxidant activities of medicinal plants.

Belonging to the family of Vitaceae, *Cissus* genus consists of 350 species and is distributed throughout the tropical and temperate areas of the world. This is one of the most widely used ingredients in alternative medicine (Ayurveda) for the treatment of piles, anorexia, indigestion, allergy, chronic ulcers, wounds and infections. They are often used as medicinal plants because they contain some bioactive compounds such as flavonoids, triterpenoids etc. which help in presenting these medicinal properties like *Cissus discolor* Blume the whole plant is crushed and ground in to a paste, mixed with egg white and applied over the wound in cattle. *Cissus quadrangularis l.* and *Cissus latifolia Lam.* is known to curebone fractures, weak bones (osteoporosis), scurvy, cancer, upset stomach, hemorrhoids, peptic ulcer disease (PUD), painful menstrual periods, asthma, malaria, and pain.*Cissus repanda Vahl.* is a folk medicine commonly known as 'Panivel' in Hindi, is a folklore medicinal herb, reputed for the healing properties of its roots and stem. Paste of the plant *Cissus repens Lam.* is applied to sloughing and fetid ulcerations, also to boils and small abscess as a maturant. Root paste and juice are given in dog bites. Ethanol (50%) extract of the plant has diuretic effect. C.*elongata* is being used by the local people for the cure of different ailments like gastrointestinal abnormalities, infections, cardiac ailments, anti-poisonous etc.

In the current work first C. *elongata* tuber extract was prepared from the ethanol, chloroform:methanol and petroleum ether. The prepared extract is used for testing different phytochemical properties of the plant. This was followed by testing antimicrobial and antioxidant properties.

MATERIALS AND METHODS

Extraction: *C.elongata* plants were collected from Udupi district and was authenticated by taxonomist Dr K G Bhat. The tubers were cleaned and were cut into pieces and shade dried for 20 days. After drying, they were powdered and subjected to successive extraction using a Soxhlet extractor. The extraction was carried out for 8-10 hours, the extract was dried under vaccum at 40˚C to get dry extracts. The solvents used for the extraction are petroleum ether, ethanol and chloroform:methanol in 3:1 ratio.

Table 1. Percentage yield of extracts in various solvents

Extraction Medium	% w/w Yield
Ethanol	10
Petroleum Ether	4.5
Chloroform:methanol(3:1)	6.5

Qualitative phytochemical screening of extracts

All the three extracts such as petroleum ether, ethanol and chloroform: methanol were qualitatively examined for various secondary metabolites using standard tests and the observations are recorded as depicted below.

Table 2. Tests for presence of different phytochemicals in the extract.

Sl.No	Group	Test	Observation	Inference
1.	Flavonoids	FeCl3 test: Test sample+few drops of 10% FeCl3 Alkaline reagent test: Test saple + 10% NaOH Lead acetate test: Test sample+ few drops of 10% lead acetate.	Blackish red color Increase in intensity of yellow color and turns colorless upon addition of dilute acid Yellow precipitate	Flavonoids confirmed
2.	Tannins	Test sample+ dil. Nitric acid Test sample+K2Cr2O7	Reddish to yellow Red precipitate	Tannins confirmed
3.	Sterols	Salkowski Test: Test sample + 2ml of chloroform + 2ml of conc. H2SO4	Chloroform layer turns red in color	Sterols confirmed
4.	Alkaloids	Hagner's test: Test sample+ saturated Picric acid	Yellow precipitate	Alkaloids confirmed
5.	Saponins	Foam test: Test sample+ water and shaken well	Formation of stable foam	Saponins confirmed
6.	Terpenoids	Salkowski Test: Test sample + 2 drops of chloroform + 2 drops of conc. H2SO4	Lower layer turns yellow	Terpenoids confirmed

DPPH (2-Diphenyle- 1 picrylhydrazyl) radical scavenging activity: Prepared plant extracts were screened for free radical scavenging activity by DPPH method. Free radical scavenging activity was carried out on the basis of scavenging activity of stable DPPH radical. Each extract at different concentrations (50,100,150,200,250µg) were added to 3ml of 0.04% DPPH in 99% ethanol and mixtures were incubated at room temperature in dark condition for 30 minutes. The scavenging activity of the extracts against DPPH radical was determined by measuring the absorbance at 517nm. Radical scavenging activity was calculated using the formula:

$\%\ \text{inhibition} = \{[(A_{control} - A_{test})/A_{control}]\} \times 100$

Where $A_{control}$ is the absorbance of the control reaction and A_{test} is the absorbance of test reaction.

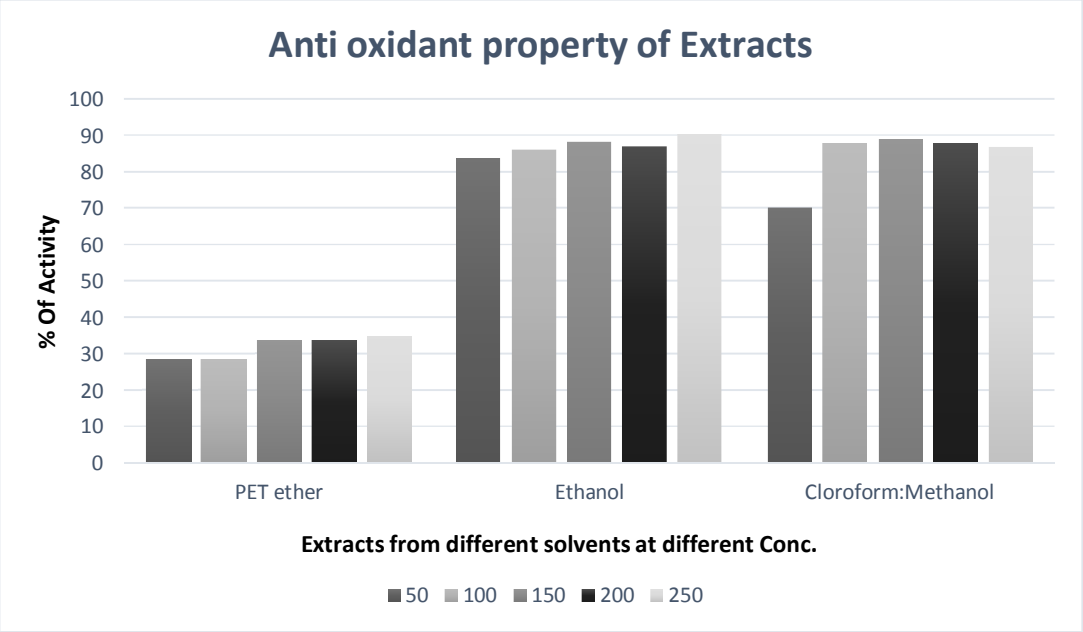

Fig. 1. Antioxidant activity of extracts of *C. elongata* for DPPH

Antimicrobial Assay

The nutrient agar and PDA media were poured into sterile petri plates and allowed to solidify. The test bacterial and fungal cultures were evenly spread over the media by sterile L shaped glass rod. Then wells (10mm) were made in the medium using sterile corkborer. 25 and 50µl plant extracts of 10mg/ml were transferred into separate wells. The standard antibiotic 25µl (fluconazole as antifungal and ciprofloxacin hydrochloride monohydrate as antibacterial) of 1mg/ml and 50µl of solvents (petroleum ether, ethanol and chloroform:methanol) were used as positive and negative control respectively. Then the plates were incubated at 37^0c for 24 hours and 72 hours for bacteria and fungi respectively. After the incubation the plates were observed for the formation of clear inhibition zone around the well indicated the presence of antimicrobial activity.The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well.

RESULTS AND DISCUSSIONS

The yield of extracts obtained from *Cissus elongata* using various solvents was analyzed (Table 1). Among the various solvents used, ethanol yielded more extractables from dried tuber, followed by Chloroform:Methanol(3:1) and Petroleum Ether. The extractables were assayed for phytochemicals qualitatively and was confirmed for presence of different phytochemicals (Table 2.). The DPPH radical scavenging ability of C. elongata extractables at different concentrations was tested. The degree of discoloration indicated the scavenging property of the extracts. At 50ppm, Petroleum Ether, Ethanol, Chloform:Methanol(3:1) showed 28.42%, 83.8%, 70.31% respectively, 28.42%, 86.02%, 87.9% for 100ppm, 33.68%, 88.17%, 89% for 150ppm, 35.63%, 87.05%, 87.9% for 200ppm, 34.7%, 90.32%, 86.8% for 250ppm respectively(Fig.1.). The solvents showed inhibitory up to some extent on the bacteria and fungus. Among the three extracts the order of inhibitory activity was Ethanol>Choloform:Methanol(3:1)>Petroleum Ether for bacteria *Staphylococcus aureus*, *Enterococcus aerogenes* and *Bacillus sphaericus*. Ethanol extract showed good antifungal activity but Petroleum Ether and Chloroform:Methanol (3:1) extract didn't show any antifungal activity for *Aspergillus niger* and *Penicillium chrysogenum*.

The results of this study have implications for the use of *C. elongata* as an antibacterial agent and moreover so as an antioxidant in several applications.

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