Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

**E-ISSN:** 2278-4136
**P-ISSN:** 2349-8234
JPP 2019; 8(3): 2342-2346
Received: 26-03-2019
Accepted: 27-04-2019

**PAM Sucharitha**
Assistant Professors,
Department of Pharmacognosy,
PRRM College of Pharmacy,
Kadapa, Andhra Pradesh, India

**SU Salma**
Department of Pharmacognosy,
PRRM College of Pharmacy,
Kadapa, Andhra Pradesh, India

**SR Mymuna**
Department of Pharmacognosy,
PRRM College of Pharmacy,
Kadapa, Andhra Pradesh, India

**SPM Ali Khan**
Department of Pharmacognosy,
PRRM College of Pharmacy,
Kadapa, Andhra Pradesh, India

**P Padmavathi**
Department of Pharmacognosy,
PRRM College of Pharmacy,
Kadapa, Andhra Pradesh, India

**PV Ramana**
Department of Pharmacognosy,
PRRM College of Pharmacy,
Kadapa, Andhra Pradesh, India

**Correspondence**
**PAM Sucharitha**
Assistant Professors,
Department of Pharmacognosy,
PRRM College of Pharmacy,
Kadapa, Andhra Pradesh, India

# Pharmacognostic, phytochemical and antimicrobial studies on ethanolic extract of aerial parts of *Cissus pallida*

**PAM Sucharitha, SU Salma, SR Mymuna, SPM Ali Khan, P Padmavathi and PV Ramana**

**Abstract**
Cissus is a genus of approximately 350 species of woody vines in the grape family Vitaceae. *Cissus pallida* is a deciduous, slender, climbing shrub. In India this species is reported to be vastly present in Maharashtra, Kolhapur, Nasik, Pune, Satara, Thane. An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoans, as well as destroying viruses. In the present study ethanolic extract of aerial parts of *Cissus pallida* was used to screen phytochemical constituents and antimicrobial activity by cup plate method. Strains of Streptomyces aureus and Escherichia coli were used to determine the antimicrobial activity. The extract showed the presence of glycosides, alkaloids, flavonoids and steroids. These constituents may be the reason for antimicrobial action. The antimicrobial activity was observed at concentration 100 µg/ml and was reported to be increased with increasing concentrations i.e., 200µg/ml, 300µg/ml and 400µg/ml, 500 µg/ml.

**Keywords:** Antimicrobial activity, *Cissus pallida*, cup plate method, ethanolic extract, Escherichia coli, Streptomyces aureus

## Introduction
Nature always stands as a golden mark exemplify the outstanding symbiosis. It is an important source for many medicinal plants. A medicinal plant also known as medicinal herb plays important role in curing and preventing of various ailments in humans. Different parts of these medicinal plants like stems, leaves, roots and fruits may have specific uses depending upon the specific constituents present in them.
The medicinal plants play an immense role in health system all over the world. Many countries in the world, that is two-third of the world's population depends on herbal medicine for primary health care. Medicinal plants have been the source for raw materials and variety of finished potent drugs which alleviate or eradicate diseases. The use of these medicinal plants have reduced the risk of side effects [1].
The trend of using medicinal herbs is increasing as they contain known and unknown chemical constituents which serve to cure numerous malfunctions in human body.

## History of medicinal plants
The medicinal plants have been in use since ancient times. The discovery and use of medicinal plants in traditional medicine proved that our ancestors were well known with the importance of herbs in human health. The earliest historical records of herbs were found from Sumerian civilization, where hundreds of medicinal plants including opium were listed on clay tablets [2]. In India Rig-veda and Atharvanaveda described the important medicinal properties of plants in curing various ailments. Ayurveda is the most ancient and Indian system of medicine and a branch of Atharvanaveda. The Indian Materia medica, wealth of India have emphasized the use of medicinal plants in human health [3]. In China many medicinal plants have been in use since 5000 B.C.
By 19$^{th}$ century the role of plants in medicine was substantially altered by the application of chemical analysis. The detection of many potent drugs in plants led to the isolation drugs by using appropriate methods. For example Alkaloids were isolated from a succession of medicinal plants, starting with morphine 1806, and soon followed by Ipecacuanha and strychnos in 1817, quinine from the cinchona tree, and then many others [4].

## Popularity of Traditional medicine
Traditional medicine is indigenous or folk medicine which makes use of traditional knowledge,

skills and practices based on theories of different cultures in society used to maintain health and prevent, diagnose, improve or treat physical and mental ailments. Traditional system of medicine has significantly developed over decades and is also the base for conventional system of medicine which is being used in this era. Ayurveda, Unani, Chinese medicine are popular traditional systems of medicine which are being practiced since prehistoric times and are rooted with different country cultures and history [5]. This traditional system started with just using plants for curing and progressed to using home remedies like cupping and leaching.

In developing countries its practice is high due to easy access and low cost whereas developed countries with best standard medical practice also are practicing this medicine because of its popularity and beliefs of people. Traditional medicine is mostly popular in rural areas because of limited availability of conventional medicine services in rural areas. Its cost effectiveness and easy availability made it attractive throughout the world [5].

**Importance of medicinal plants in human health**

The thought of world without plants is just unimaginable because of their incredible role in our human lives. The air we breathe and food we eat are just main instances of what mankind is blessed by plants. Similarly the discovery of medicinal plants by early humans has a marked effect on human health. They are the primary source of health care all across the world. The invention of allopathic medicine did not stop the use of medicinal plants because of their no or minimal side effects.

Based on a report the number of medicinal plants found are 30000 and among them as many as 15000 plants are being used worldwide as potent drugs. Millions across the globe use plants to satisfy their emergency or day to day healthcare needs [3]. The isolation of components with medicinal properties from plants like Alkaloids, Glycosides, Resins, Tannins, flavonoids, aminoacids etc paved the way for the discovery of potent drugs. The constituents from plants have different impact on human body. Medicinal plants are being used from treating simple cuts or wounds to combating several heart, lung, liver, and kidney diseases.

**Table 1:** Common medicinal plants with their constituents and uses [6]

| S. No | Name of plant | Chemical Constituent | Medicinal uses |
|---|---|---|---|
| 1 | Digitalis | Digitoxin | Congestive heart failure |
| 2 | Ashoka | Catechol | Oxytocic |
| 3 | Physostigma | Physostigmine | Cholinergic in Glaucoma |
| 4 | Vasaka | Vasicine | Antitussive & Expectorant |
| 5 | Amla | phyllemblin | Diuretic |
| 6 | Ashwagandha | withanine | Immunomodulator |

**Antimicrobials**

Many living organisms coexist on earth which are useful as well as harmful in nature. Microorganisms are perfect instances for providing humans with both pros and cons. They are useful in manufacturing several food products like cheese and many other dairy products. Similarly they have been causing chaos all over the world by infecting humans with number of diseases like jaundice, HIV, TB, cholera, gonorrhea, syphilis, pneumonia etc. With the advent of newer microorganisms many dreadful diseases such as Ebola are creating a threat to mankind. So for this purpose many useful antimicrobials are in urgent need. The invention of antimicrobials is the greatest blessing to mankind. Antimicrobials are the substances which kill the microorganisms or prevent the spread of microorganisms by suppressing their growth. The substances which kills the microbes are termed as microbiocidals and those which suppress the growth of microbes are termed as microbiostatics. Antimicrobials include antibiotics, antifungals, antiviral and antiprotozoal agents which differ in treating different type of infections caused by different organisms such as bacteria, fungi, virus and protozoa respectively.

As number of microorganisms are emerging in this era, antimicrobials are the only hope to eradicate microbes and the diseases caused by them. But some powerful microbes are gaining resistance against antimicrobials due to one of following reasons: [7]

1. Natural resistance by certain type of microorganisms
2. Genetic mutations
3. Or by acquiring resistance from another species

For this purpose tremendous researches and inventions of antimicrobials are in need to discover potent antimicrobials for the resistant microbes as well as newly emerging microorganisms.

**Cissus**

*Cissus* is a genus of approximately 350 species of woody vines in the grape family Vitaceae. They have a cosmopolitan distribution and majority are found in tropics. The generic name Cissus is derived from greek word "*kissos"* meaning "*ivy".* This genus name was given by Carl Linnaeus [8].

***Cissus pallida***

*Cissus pallida* is also commonly called as entire leaf wild grape which is a climbing shrub producing 5-15cm long slender stems. This plant is deciduous meaning the shrub tends to shed leaves or petals or ripened fruits at the end of season [9].

**Plant profile**

Botanical Name: *Cissus adnata*

Synonyms: *Cissus pallida, Vitis simplex, Vitis adnata, Cissus compressa* [9]

Family: *Vitaceae*

**Vernacular names in India**

Telugu: Gudamathige
Tamil: Nanaminukki [10]
Kannada: Gudametake
Malayalam: Nadena
Common Name: Entire-leaf wild grape

**Scientific classification [11]**

Kingdom: plantae
Class: Angiosperms
Order: Vitales

Family: Vitaceae
Genus: *Cissus*
Species: *Cissusadnata*

**Geographical distribution**
It is vastly present in many parts of India such as Maharashtra, Kolhapur, Nasik, Pune, Satara, Thane [10], Himalaya, from Garhwal to North East India, Western Ghats of South India and Niligiri hills. It is also found in Australia and in Malaysia it grows at an altitude of 800-1100m [9].

**Morphological features of *Cissus pallida***
*Cissus pallida* is a climbing shrub and have slender long stems ranging from 5-15 cm. Its flowers are red in colour and borne in umbels, leaf-opposed on flower cluster stalk which is 1.5-4.5 cm with densely rusty curly hairs. Flowers are 4-merous. They flower from june to august. Flower stalks are velvet to hairy ranging from 1.5-2.5 mm. The buds are oval, 2 mm long and tip of buds is rounded or blunt. Calyx is wavily lobed and velvet-hairy. Petals are oval, ranging from 1.3-1.7 mm long, velvet-hairy. Petals are distinct, greenish-yellow in colour which are less than 1mm long in oblong-ovate shape, hooded and velvet-hairy outside. Style is columnar. Ovary is sparsely hairy,style is conical and stigma is expanded. The stems either scramble over the ground or climb into surrounding vegetation for support, attaching themselves by means of tendrils bifurcate. Leaves are simple, both surfaces are of same color when they are dry. Leaf stalk ranges from 1.5-7cm long and is densely rusty hairy. leaf blade is heart shaped to oval with measurements ranging from 6-11.5 x 5.5-8.5 cm. Basal veins are 3-5 and lateral veins are of 5 or 6 pairs. Leaf margin is serrate- crenate and has 35-40 sharp teeth on each side. Leaf apex is acuminate. Berry is 6-7 x 5-6 mm and is 1-seeded. Seed surface is smooth with sharp ridges, ventral holes short and narrow [9, 10].

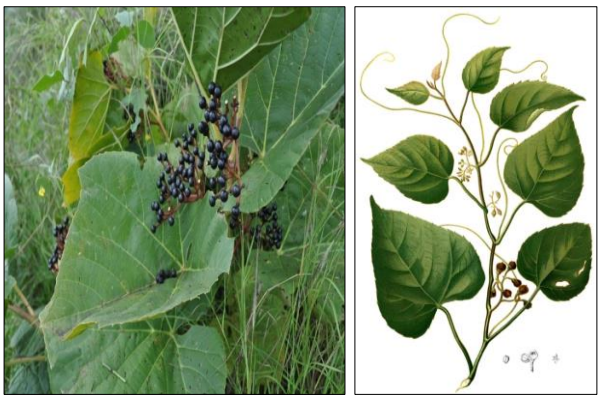


**Fig 1:** *Cissus pallida*

**Materials and methods**
**Plant collection**
The plant *Cissus pallida* was collected from the local area of Kadapa district. The collected species were authenticated by a renowned Botanist Dr. A. Madhusudhana Reddy Asst. professor, Department of Botany, Yogi Vemana University, Kadapa.

**Extraction**
**Preparation of extracts**
About 90 gm of the shade dried aerial parts of powdered plant material was reduced to moderately coarse powder and extracted by Soxhlet apparatus [12] using ethanaolic solvent.

**Phytochemical analysis**
The ethanolic extract was subjected to systematic qualitative phytochemical screening to find out the presence or absence of phytoconstituents [13] like carbohydrates, glycosides, alkaloids, flavonoids etc.

**Anti microbial activity**
Strains of *Streptomyces aureus*, *Escherichia coli* were used for the study. Streptomycin was used as the standard drug. The inhibition of growth of microorganisms was measured by using concentration of the compound/test extract to be examined.

**Antimicrobial studies**
**Preparation of culture**
The media used for the growth of micro organism was
1. Nutrient Agar Medium

**Nutrient agar medium**
The nutrient agar medium was prepared as per the following formula
Peptone – 1%
Sodium chloride – 0.5%
Beef extract – 1%
Agar – 2%
pH – 7.4

**Method of preparation**
For the preparation of Nutrient agar medium, 10g of Beef extract. 5g of Sodium chloride and 10g of peptone were accurately weighed and transferred to a conical flask. To it 500ml of distilled water was added, stirred well to dissolve the contents and heated. In boiling condition, 20g of agar was added with constant stirring and heated till agar dissolves completely. $P^H$ of media was adjusted to the range of 7.0 – 7.5 by either using Sodium hydroxide (1N) or Hydrochloric acid. Then the nutrient agar medium was sterilized by autoclave at 15 Ib/sq inch at $121^0$ C for 15min. The microbial assay may be carried out by cup plate method.

**Cup Plate method**
The antimicrobial activity of the extract was determined by using agar well diffusion technique. Agar plates were seeded with 0.1ml of overnight allowed culture and were allowed to incubate for 24hrs. Cups were made in petri plates and 50 microlitre of each extract was added into each well and plates were incubated at 37°C 24 hrs. The antimicrobial activity was determined by measuring zone of inhibition around each plate.

**Results and discussion**
**Pharmacognostical analysis**
The present study on pharmacognostical characteristic of *Cissus pallida* provided important information to its identity and helped to differentiate it from the closely related other species.

**Ash value**
For determination of ash value, shade dried powdered plant material was passed through sieve no:40. Total ash, acid insoluble ash and water soluble ash *Cissus pallida* of was determined by standard method and the results are tabulated in Table 2.

**Table 2:** Ash values

| Type of ash value | *Cissus pallida* |
|---|---|
| Total ash in %w/w | 14.01% w/w |
| Acid insoluble ash in %w/w | 9.42% w/w |
| Water soluble ash in %w/w | 9.42% w/w |

**Results of moisture content**

The moisture content (loss on drying) of *Cissus pallida* was determined by standard method and the results are tabulated in Table 3.

**Table 3:** Moisture Content

| Plant name | Amount of moisture % w/w |
|---|---|
| *Cissus pallida* | 8.91% |

**Results of Extraction**

The percentage yield of extraction of *Cissus pallida* were determined by soxhlet method by using different solvents and the results are tabulated in Table 4.

**Table 4:** Percentage yield of Extraction.

| S. No | Solvent | Percentage yield |
|---|---|---|
| 01. | Water | 14%w/w |
| 02. | Ethanol | 10%w/w |
| 03. | Chloroform | 6%w/w |
| 04. | Methanol | 9%w/w |

**Qualitative Preliminary phytochemical studies**

The ethanolic extract was subjected to qualitative preliminary phytochemical tests to identify the various phytoconstituents.

**Table 5:** Qualitative phytochemical studies of ethanolic extract of *Cissus pallida*

| Test | Ethanolic Solvent |
|---|---|
| **Carbo Hydrates** | |
| Molisch Test | + |
| Fehling's Test | + |
| Benedict's Test | + |
| Seliwanoff's Test | _ |
| **Alkaloides** | |
| Dragendroff's Test | + |
| Wagner's Test | - |
| Mayer's Test | + |
| Hager's Test | + |
| **Glycosides Cardiac Glycoside** | |
| Legal's Test | + |
| Keller Kiliyani Test | + |
| Libermanns Test | + |
| **Tannins** | |
| 5% Fecl3 | + |
| Acetic Acid | + |
| Iodine Solution | + |
| Lead Acetate | + |
| **Steroids** | |
| Salkowski Reaction | + |
| Libermann-Burchards Test | + |
| **Falvonoids** | |
| Shinoda Test | + |
| Lead Acetate | + |

**+ =** Present and −= Absent

**Antimicrobial activity**

1 The ethanolic extract of aerial parts of *Cissus pallida* was used to screen the antimicrobial activity. The study gave positive results for antimicrobial activity.
2 The antimicrobial studies were carried out by the cup-plate technique.

The plant extract was tested for anti microbial activity at increasing concentrations of 100, 200, 300, 400, and 500µg/ml. *Streptomycin* was used as a standard for the antimicrobial activity. Results are tabulated in Table 6.

**Table 6:** Antimicrobial Activity (Zone of Inhibition) of ethanolic extract of *Cissus pallida*

| S. No | Test Pathogen | Zone Of Inhibition | | | | | |
|---|---|---|---|---|---|---|---|
| | | Volume Of Extracts | | | | | Streptomycin |
| | | 100µg/ml | 200µg/ml | 300µg/ml | 400µg/ml | 500µg/ml | 300µg/ml |
| 01 | Escherichia coli | 0.9 mm | 1.1 mm | 1.1 mm | 1.2 mm | 1.3 mm | 1.7 mm |
| 02. | Streptomyces aureus | 0.8 mm | 0.8 mm | 0.9 mm | 1 mm | 1.1 mm | 1.5 mm |

µg/ml – microgram/ millilitre
mm – micrometres

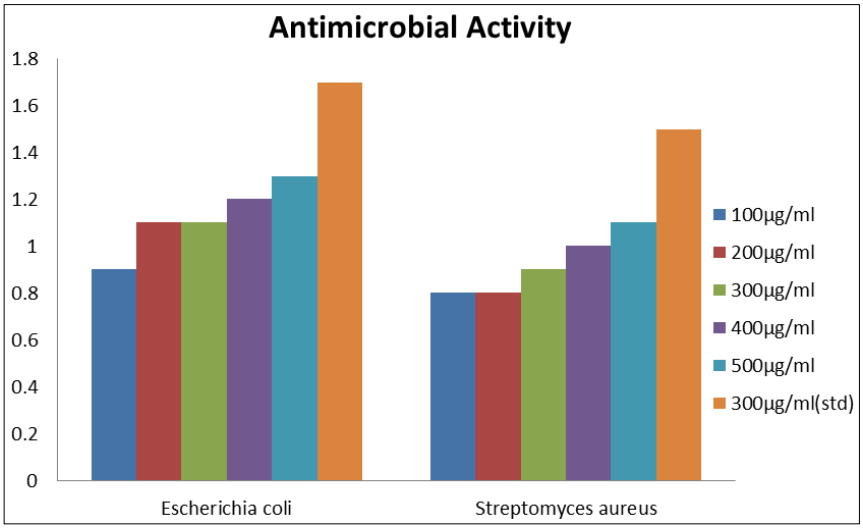
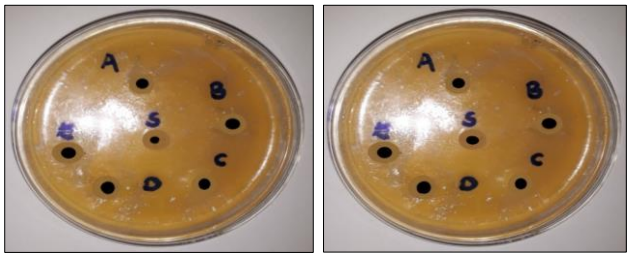



**Fig 2:** Antimicrobial Activity




**A.** **B.**

**Fig 3:** Antimicrobial activity of *Cissus pallida* against
**A.** Escherichia coli
**B.** Streptomyces aureus

## Conclusions

The pharmacognostical studies on *Cissus pallida* provided useful data for the correct identification of the plant. The plant was extracted with ethanolic solvent of increasing polarity. The obtained extract was screened for different phytoconstituents. The extract showed very good reaction for carbohydrates, alkaloids, glycosides, tannins, steroids, flavonoids, etc. This may be the reason for the very good antimicrobial activity. So this extract is taken for investigating the anti microbial activity of the plant. The plant is claimed to be useful in treatment of wounds and skin diseases in traditional medicine and upon scientific investigations the antimicrobial activity of the plant was proved to be correct. The plant is screened for its antimicrobial activity. The experimental results suggests that *cissus pallida* has potential antimicrobial properties and the activity increases with increasing concentration of plant extract. The broad spectrum antimicrobial activity of the plant extract, is possibly due to the identified phytoconstituents.

## Acknowledgement

We are thankful to management of P. Rami Reddy Memorial College of Pharmacy, Kadapa, Andhra Pradesh for providing all facilities to carry out this work.

## References

1. Bhat KKP. Medicinal plant information databases. In: Non-Wood forest products. Medicinal plants for conservation and health care, Rome: food and agriculture organization, 1995.
2. www.google.com[homepage],https://en.wikipedia.org/wiki/Medicinal_plants#cite_note-footnote Petrovska2012-33, Wikipedia of Medicinal plants.
3. Manoharachary C, Nagaraju D. Annals of Phytomedicine, Medicinal plants for human health and welfare. 2016; 5(1):24-34.
4. Atanasov AG, Waltenberger B, ferschy-Wenzig PEM, Linder T, Wawrosch C, Uhrin P *et al*. Discovery and resupply of pharmacologically active plant-derived natural products: A review. Biotechnol Adv. 2015; 33(8):1582-614. doi:10.1016/j.biotechadv.2015.08.001. PMC 4748402. PMID 26281720.
5. https://www.who.int/traditional-complementary-integrative-medicine/publications/trm_strategy14_23/en/, available from WHO Traditional Medicine Strategy 2014-2023
6. Kokate CK, Purohit AP, Gokhale SB Pharmacognosy, 51st edition, Nirali Prakashan, Pune, 2015.
7. www.tufts.edu, General Background: About Antibiotic Resistance Archived from the original on 23, 2015. Retrieved 30 October 2015.
8. https://en.wikipedia.org/wiki/Cissus
9. https://www.flowersofindia.net/catalog/slides/Entire-Leaf%20Wild%20Grape.html
10. Flora of Kolhapur District, Yadav S. R & Sardesai M. M, 2002, Flora of Maharastra State Dicotyledones, Lakshminarasimhan P. &Prasanna P. V, 2000, I.
11. Cissus Adnata. Australian Plant Name Index (APNI), IBIS database. Centre for Plant Biodiversity Research, Australian Government, Canberra, 2013. Retrieved 20.
12. Harbone JB. Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis, London, Chapman and Hall, 1998, 62-68.
13. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy, 3rd edition. NiraliPrakashan, Pune, 1995, 290-298.