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Phytochemical Screening for Secondary Metabolites of *Opuntia Cochenillifera* (L.) Mill

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Abstract: ***Opuntia cochenillifera*** **is a commonly grown cactus in India; however, no reports are available on its Phytochemistry and ethno pharmacology from India, So this is the First report on this plant species. Therefore, the present study was undertaken to evaluate the phytochemical constituents in cladodes and fruits. Cladode showed the presence of Phenols, Alkaloids, Flavonoids, Saponins, Tannins, and Resins. While, fruits showed the presence of Phenols, Alkaloids, Flavonoids, Terpeonids Steroids, Saponins, Tannins, and Resins Among the phytochemical detected in cladode Flavonoids (5.8%) were found to be maximum followed by phenols (4.1%), Alkaloids (2.05%), Saponins (1.08%), Tannins (0.9%). Similarly, in the fruit, Flavonoids were found maximum (6.1%) followed by Phenols (3.38%), Alkaloids (1.75%), Saponins (1.22%), Tannins (1.2%) and Terpenoids (0.8%).**

Keywords: **Phytochemical, Secondary metabolites, Cladodes, Fruits,** ***Opuntia Cochenillifera.***

1. INTRODUCTION

In the ancient India, medicinal plants were used to prevent various critical diseases. The plant kingdom is an important source of herbal drugs. Even in recent years, there has been an increasing awareness about the importance of medicinal plants. Generally, herbal drugs are easily available, safe, less expensive, efficient, and rarely have side effects. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs[1] Herbal drugs play a role in the management of various disorders; most of them speed up the natural healing process of humans. Numerous medicinal plants and their formulations are used for various disorders in ethnic medical practices as well as a traditional system of medicine in India. Since pre-historic days attempts are being made to find out suitable drugs from natural sources for treatment of different diseases. Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include alkaloids, flavonoids, phenols, tannins, saponins, glycosides, resins, terpenoids, and steroids. The bio-active phytocompounds are synthesized by primary or rather secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy.

Opuntia is distinguished from all other cactus genera by having flattened joints or pads called cladodes. The young pads produce small leaves that quickly fall away. Most *opuntia* has typical cactus spines and all have glochids, which are tiny hair like spines that are usually easily dislodged from the plant. Fruits are edible it promotes many health benefits.

Opuntia cochenillifera also called as Nopalea *Cochenillifera* means 'cochineal-bearing (an edible red dye)' specific to this cactus. The cochineal cactus provides food for the cochineal insect that itself is the source of the red cochineal dye. The plant has edible fruits and stems, which are gathered from the wild for local use. In the past, the plant was often grown as a host plant for the cochineal insects (*Dactylopius coccus*) these insects were then harvested, killed and made into the red dye cochineal. Synthetic dyes have almost totally usurped this use, though the plant is still often cultivated, but only as an ornamental and hedge plant

Plant is native to Mexico or Central America. They are a good source of fruits and vegetables for human consumption, as well as fodder for cattle and other animals during the dry seasons. They are also used for medicinal purposes, in cosmetics, to produce dyes and as natural fences [1-3]. They are commonly consumed as a fresh or cooked green vegetable in Mexico and in some parts of the United States[4,5] This species is for the most part free of spines and spine-hairs[6-7]. There are few reports on the biological activity of Nopalea species. These plants have been used to treat rheumatism and other inflammatory problems and diarrhea, as well as a diuretic and analgesic, particularly for ear and tooth aches[8-12]. The anti-inflammatory principle was related to a b-sitosterol[13]. *Nopalea cochenillifera* was also shown to inhibit herpes simplex virus type 1 infection [14].

2. MATERIALS AND METHODS

Plant material:

Collection of raw materials:

The *Opuntia cochenillifera* were collected from Gulbarga University campus. The Plant species were identified with the help of the Digital flora of Karnataka, Flora of Presidency of Madras, Flora of Gulbarga district and the Flora of Karnataka[15-17] The cladodes and fruits were shade dried, powdered mechanically and stored in airtight containers for extraction.

Preparation of crude extracts:

The powdered materials *Opuntia cochenillifera* were subjected to successive solvent extraction. The powdered were taken separately in 1l capacity thimble of soxlet apparatus and refluxed successively with petroleum ether, chloroform, ethyl acetate, methanol and aqueous for 48h in 8 batches of 500g each. Each time, the solvent from the mark was removed completely before extracting with the next solvent. And allow for evaporating the crude extracts so been used for phytochemical analysis.

Phytochemical screening:

The preliminary phytochemical studies were performed for testing the different chemical groups present in petroleum ether, chloroform, ethyl acetate and methanol extracts of *Opuntia cochenillifera*.

Test for Alkaloids:

A. Dragendorff's test: To 2 mg of the extract 5 ml of distilled water was added, 2ml Hydrochloric acid was added until an acid reaction occurs. To this 1 ml of Dragendorff's reagent was added. Formation of orange or orange red precipitate indicated the presence of alkaloids.

B. Hager's test: To 2 mg of the extract taken in a test tube, a few drops of Hager's reagent were added. Formation of a yellow precipitate confirmed the presence of alkaloids.

C. Wagner's test: 2 mg of the extract was acidified with 1.5 % v/v of hydrochloric acid and a few drops of Wagner's reagent were added. A yellow or brown precipitate indicated the presence of alkaloids[18]

Test for Phenols:

Ellagic Acid Test: The test solution was treated with a few drops of 5% (w/v) glacial acetic acid and 5% (w/v) $NaNO_2$ solution. The solution turned muddy or Niger brown precipitate occurred in the extract. It indicates the presence of phenol solution.

Ferric chloride test: 0.5 ml of $FeCl_3$ (w/v) solution was added in 2 ml of test solution, formation of an intense colour indicates the presence of phenols[19]

Hot water test: Deep the mature leaf in a beaker containing hot water, warm it for a minute development of black or brown colour ring at the junction of Deeping indicates the presence of phenols.

Test for Flavonoids:

A. Shinoda's test: In a test tube containing 0.5 ml of the extract 10 drops of dilute hydrochloric acid followed by a small piece of magnesium were added. Formation of pink, reddish or brown colour indicated the presence of flavonoids.

B. Ferric chloride test: Test solution with a few drops of ferric chloride solution shows intense green colour.

C. Zinc-Hydrochloric acid reduction test: Test solution with zinc dust and a few drops of hydrochloric acid shows magenta red colour.

D. Alkaline reagent test: Test solution when treated with sodium hydroxide solution, shows an increase in the intensity of yellow colour which becomes colourless on addition of a few drops of dilute acid.

E. Lead acetate solution test: Test solution with a few drops of lead acetate (10%) solution gives a yellow precipitate.

Test for Triterpenoids:

A. Liebermann - Burchard's test (LB test): 2 mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of a violet coloured ring indicated the presence of triterpenoids.

B. Salkowaski test: When a few drops of concentrated sulphuric acid were added to the test solution, shaken and allowed to stand, lower layer turns yellow indicating the presence of triterpenoids.

Test for Saponins:

A. Foam test: In a test tube containing about 5 ml of extract, a drop of sodium bicarbonate solution was added. The test tube was shaken vigorously and left for 3 minutes. Formation of honeycomb like froth indicated the presence of saponins.

Test for Steroids:

A. Liebermann-Burchard's test: 2 mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of green colour indicated the presence of steroids.

B. Salkowaski reaction: 2 mg of dry extract was shaken with chloroform, to the chloroform layer sulphuric acid was added slowly by the sides of the test tube. Formation of red colour indicated the presence of steroids.

C.Sulphur test: pinch of sulphur powder added to test solution it sinks to the bottom it indicates the presence of steroids

Test for Tannins:

A. Ferric chloride test: To 1-2 ml of the extract, few drops of 5% w/v $FeCl_3$ solution were added. A green colour indicated the presence of gallotannins, while brown colour indicated the presence of pseudotannins.

B. Gelatin test: Test solution when treated with a gelatin solution gives white precipitate.

colour This confirmed the presence of a naphthoquinon[20]

Test for glycosides:

A. Keller-Killiani test:

The test solution was treated with a few drops of ferric chloride solution and mixed. When concentrated sulphuric acid containing ferric chloride solution was added, it forms two layers, lower layer reddish brown and upper acetic acid layer turns bluish green.

B. Legal's test: Test solution when treated with pyridine (made alkaline by adding sodium nitroprusside solution) gives pink to red colour.

Test for Resins:

1 ml of extract was dissolved in acetone and the solution was poured in distilled water. Turbidity indicated the presence of resins.

Quantitative estimations of secondary metabolites:

Alkaloids [21] Flavonoids [22] Tannin [23] Phenols [24-25]Saponins [26]Terpenoids[27] were estimated by standard methods

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3. RESULT AND DISCUSSION

These reactions are generally simply identical, quick to implement, performed mostly in test tubes, they appear either mowing staining or precipitation, which can give an idea, according to the intensity of the result, the concentration of certain constituents. The results obtained from of *Opuntia cochenillifera* are represented in the below table.

Table 1: Phytochemical results obtained from *Opuntia cochenillifera* from different solvents by cladode and fruit

Phytochemical Constituents	Petroleum ether		Chloroform		Ethyl acetate		Methanol		Aqueous	
	Cladode	Fruit	Cladode	Fruit	Cladode	Fruit	Cladode	Fruit	Cladode	Fruit
Alkaloids										
Dragendorff's test	+	+	+	+	+	+	+	+	-	-
Hager's test	+	+	+	+	+	+	+	+	-	-
Wagner's test	+	+	+	+	+	+	+	+	-	-
Phenols										
Feric chloride test	+	+	+	+	+	+	+	+	+	+
Ellagic acid test	+	+	+	+	+	+	+	+	+	+
Hot water test	+	+	+	+	+	+	+	+	+	+
Flavonoids										
Shinoda test	+	+	-	-	-	-	+	+	-	-
Ferric chloride test	+	+	+	+	+	+	+	+	+	+
Zinc Hydrochloric acid reduction test	-	-	-	-	+	-	+	+	-	-
Alkaline reagent test	+	+	+	+	+	+	+	+	+	+
Lead acetate test	-	+	-	+	-	-	+	+	+	+
Terpinoids										
Liebermann Burchard's test (LB test)	-	+	-	+	-	+	-	+	+	+
Salkowaski test	+	+	-	+	-	+	+	+	+	+
Saponins										
Foam test	+	+	+	+	+	+	-	-	-	-
Steroids										
Liebermann - Burchard's test (LB test	-	-	-	-	-	-	-	-	+	-
Salkowaski test	-	+	-	+	-	+	-	+	-	+
Sulphur Test	+	+	+	+	+	+	+	+	-	-
Tannins										
Ferric chloride test	+	+	+	+	+	+	+	+	-	-
Gelatine test	+	+	+	-	+	+	+	-	+	+
Glycosides										
Baljet test	-	+	-	-	+	-	-	-	-	-
Keller-Killiani test	-	-	-	-	+	-	+	-	-	-
Raymond's test	-	-	-	-	+	-	+	-	-	-
Bromine water test	-	-	-	-	+	-	+	+	+	+
Legal's test	-	-	-	-	+	-	+	+	-	-
Resins	+	+	+	+	+	+	+	+	-	-

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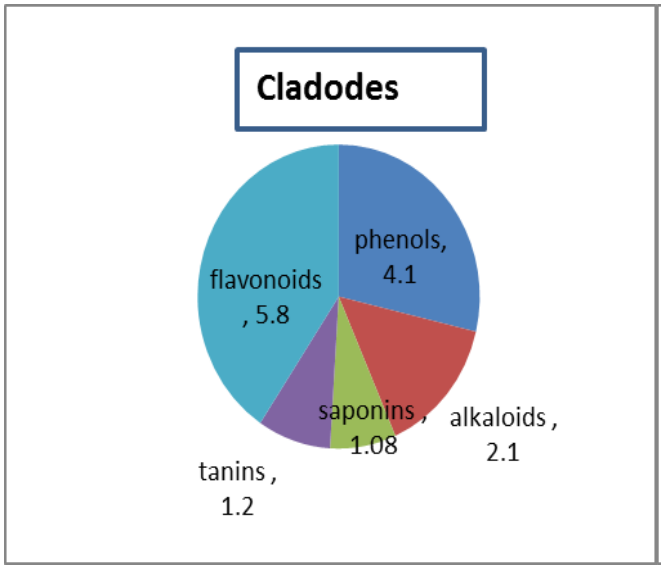

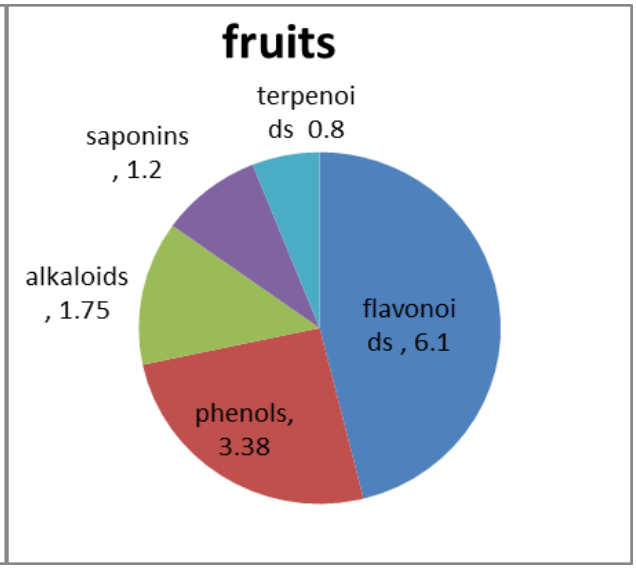

Fig:1 **Fig:2**

Fig:1 Quantitative estimation of secondary metabolites of cladodes

Fig: 2 Quantitative estimation of secondary metabolites of Fruits

Result and discussion:

Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities[28] Analysis of the cladode extracts revealed the presence of Phenols, Alkaloids, Flavonoids, Saponins, Tannins, and Resins. While, fruits showed the presence of Phenols, Alkaloids, Flavonoids, Terpeonids Steroids , Saponins, Tannins, and Resins Among the phytochemical detected in cladode Flavonoids (5.8%) were found to be maximum followed by phenols (4.1%), Alkaloids (2.05%), Saponins (1.08%), Tannins (0.9%).(fig :1) Similarly, in the fruit, Flavonoids were found maximum (6.1%) followed by Phenols (3.38%), Alkaloids (1.75%), Saponins (1.22%), Tannins (1.2%) and Terpenoids (0.8%) (fig:2)Several studies has described the antioxidant properties of different parts of various medicinal plants which are rich in phenolic compounds [29-30]

Alkaloids have different types of activities as painkillers, antimicrobial, stimulants, muscle relaxants, anaesthetics, anti-microbial, anti-diabetic, anti-cancerous, anti-HIV, antioxidants, etc. Flavonoids have an inherent ability to modify the body's reaction to allergen, virus and carcinogens. They show anti-allergic, antimicrobial and anticancer activity by which Tannins have general antimicrobial and antioxidant activities[31] Current reports show that tannins may have potential value such as cytotoxic and antineoplastic agents [32] Saponins have antifungal properties [33] These contents show different types of activities against different pathogens. Therefore, it can be used in the treatment of diseases. Saponins are used in hypercholesterolemia, hyperglycemia, antioxidant, anticancer, anti-inflammatory and weight loss etc. according to medical field. It is bioactive antibacterial agents of plants [34-35] Plant steroids have cardiotonic activity, possess insecticidal and antimicrobial properties. It is generally used in herbal medicines and cosmetic products [36] Phenolic compounds have anti-oxidative, antidiabetic, anticarcinogenic, antimutagenic and anti-inflammatory.[37-38] Thus, these preliminary qualitative tests according to[39] is useful in the detection of bioactive principles and subsequently may lead to drug discovery and development.[40]

4. CONCLUSION

The purpose of the study was to find out the preliminary phytochemical screening of the extract of cladode and fruits *Opuntia Cochenillifera*. Flavonoids and tannins compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers. Since these compounds were found to be present in the extracts, it might be responsible for the potent antioxidant capacity of in future it may be an agent for treating oxidative stress related disease along with microbial infections.

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