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## Phytochemical screening for secondary metabolites of *Opuntia dillenii* Haw

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### Abstract

*Opuntia dillenii* a wild xerophyte is abundant in Himalayas, believed to be of American origin and a native of India. Traditionally, the plant is used in the treatment of inflammation, hypoglycaemic, stomach ulceration, Neuro-protection through antioxidant action, viral disease, diabetes, burns, bronchial, asthma and digestive problems throughout the world. Phytochemistry study was undertaken in cladodes and fruits and revealed the presence of Phenols, Alkaloids, Flavonoids, Saponins in cladode and Phenols, Alkaloids, Flavonoids, Terpenoids Steroids, Saponins in fruit. Phenols content (6.8%) was found maximum followed by Alkaloids (5.4%), Flavonoids (3.5%) and Saponins (1.05%) in cladode. Similarly, in the fruit, Phenols were maximum (5.4%) followed by Alkaloids (4.38%), Flavonoids (2.22%), Saponins (0.75%), and Terpenoids (0.8%). Steroids were detected in fruit.

**Keywords:** Phytochemicals, secondary metabolites, cladode, fruits alkaloids

### Introduction

Medicinal plants contain some organic compounds which provide definite physiological action on the human body. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy [1] Secondary metabolites are the substances produced by plants as defense chemicals. They include alkaloids, flavonoids, essential oils, phenols, saponins etc. Recently, many pharmaceutical companies have spent a lot of time and money in developing natural products extracted from plants, but the information available is quite meagre [2]. The present study was carried out to identify the active chemical principle composition in *Opuntia dillenii*

*Opuntia dillenii* (Ker-Gawl) Haw family Cactaceae commonly known as pear bush, prickly pear, mal rchette or tuna, is a succulent shrub growing under desert and dry conditions. It is native to the American continent and the West Indies, but recently due to cultivation, it has become widely distributed throughout Canary Islands, Southern and Eastern Africa, Pakistan, India and Australia [3,4] *Opuntia dillenii* is a rich source of dietary fibres, natural colorants and antioxidant vitamins and therefore, used as a food because of their edible fruit [5] Pharmacological evaluation of *Opuntia* has shown its efficacy as antihyperlipidemic, [6] antiviral [7] anti-inflammatory [8] antidiabetic [9] antioxidant and antiulcerogenic activity [10]. It has also been reported to protect nerve cells and used for the treatment of Alzheimer's disease, Parkinson's disease and stroke [11]. In recent years, there has been a global trend toward the use of natural resources as antioxidants and functional foods [12]. Two characteristic historical examples are the *O. dillenii* plantations of Srirangapatam (India). In the first case, the Ruler of Mysore, Tippu Sultan (1750-1799), reinforced the fortification around his residence with the cactus because of its formidable spines [13]. Secondly, in 1930 the Imam established the cactus near his castle in order to use the purple coloured fruit juice as ink [14].

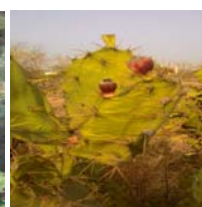
### Plant habit



**Fig 1:** Habit



**Fig 2:** Flower



**Fig 3:** Fruits

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## Materials and methods

### Collection Plant material

The *Opuntia dillenii* 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. <sup>[15-17]</sup>.

### Preparation of crude extracts

The powdered materials of *Opuntia dillenii* were subjected to successive solvent extraction using Soxhlet apparatus and refluxed successively with petroleum ether, chloroform, ethyl acetate, and methanol and aqueous for 48 h in 8 batches of 500g each. The crude extracts were allowed for evaporation and used the deposited crude solid material for phytochemical analysis.

### Phytochemical screening

#### Test for Alkaloids

**A. Dragendorff's test:** To 2 mg of the extract 5 ml of distilled water was added, 2M 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 <sup>[18]</sup>.

#### 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<sub>2</sub> 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<sub>3</sub> (w/v) solution was added in 2 ml of test solution, formation of an intense color indicates the presence of phenols <sup>[19]</sup>

**Hot water test:** Deep the mature plant part 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 the 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 colored ring indicated the presence of triterpenoids.

**B. Salkowski 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. Salkowski 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<sub>3</sub> 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 naphthoquinone <sup>[20]</sup>

#### 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.

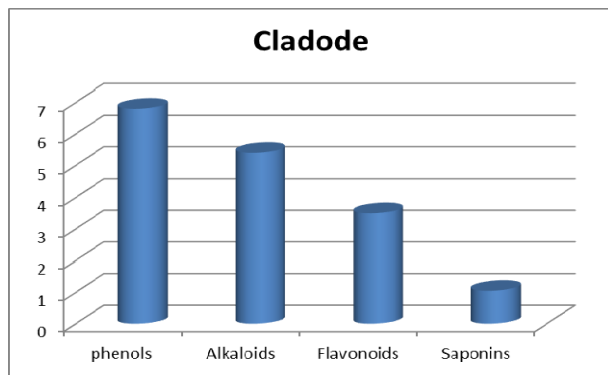
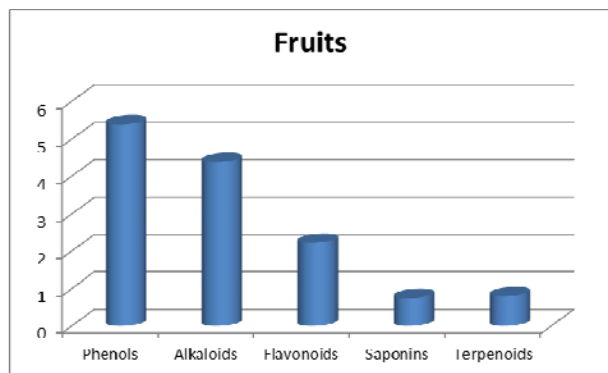
#### Quantitative estimations of secondary metabolites

Alkaloids <sup>[21]</sup> Flavonoids <sup>[22]</sup> Phenols <sup>[23]</sup> Saponins <sup>[24]</sup> terpenoid <sup>[25]</sup> were estimated by using the standard methods.

## Results and Discussion

**Table 1:** Phytochemical result obtained from *O. dillenii* cladode and fruits in different solvent extracts

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

**Fig 4:** Quantitative estimation of secondary metabolites of Cladode mg/100g**Fig 5:** Quantitative estimation of secondary metabolites of Fruits mg/100g

Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities [26]. Analysis of the cladode extracts revealed the presence of Phenols, Alkaloids, Flavonoids, Saponins, and glycosides, while fruits showed the presence of Phenols, Alkaloids, Flavonoids, Terpenoids Steroids, Saponins and Tannins. Among the phytochemicals detected in cladode, phenols (6.8%) were found to be maximum followed by Alkaloids (5.4%), Flavonoids (3.2%), Saponins (0.8%) (Fig-4). Similarly, in the fruit, Phenols were maximum (5.2%) followed by Alkaloids (4.3%), Flavonoids (1.8%), Saponins (0.9%), and Terpenoids (0.6 %) (Fig-5).

Alkaloids and phenols were present in all the extracts except phenols in aqueous extract. Terpenoids are absent in cladode of all extracts. Though aqueous extract is a rich source of many phyto constituents, methanolic extract seems to be better as it has all the phytoconstituents. Alkaloid, saponins are present exclusively in all extract. Same way, flavonoids are present in all extract. Compared to other phytoconstituents. Saponins and tannins, missing in all extract and resins was found absent in all the extracts.

Several studies have described the medicinal importance of *O. dillenii* through folk medicine of China Algerian Nigeria countries. For instance India, where it is called Kanthari or Nagphana in Hindi [27]. *O. dillenii* is obviously very reputable as indicated by a compilation of literature on its pharmacological properties [28] and the preparation of several health drinks from this plant [29-31]. Plant phenols are highly potent ROS scavengers and among them the flavonoids are most prominent [32]. The effects shown by *O. dillenii* in disease

models certainly arise from the various properties of cactus components. Carrageenan-induced inflammation was inhibited by aqueous and alcoholic extracts from fruits [33] and cladodes, flowers and fruits [34]. The residue of a methanolic extract from *O. dillenii* cladodes fed to male rats (250 mg/kg body weight) for 60 days reveals a significant reduction in weight and changes in the structure of the genital organs with a total loss in fertility. The effects could be attributable to the detected flavonoids [35]. Consequently, *O. dillenii* is a highly promising candidate of programmes directed to the development of *Opuntia* species into crop plant [36]. As reported above several of these secondary metabolites occur in *O. dillenii* and they may contribute a great deal to the antioxidant activity of a different solvent extract obtained from cladodes and fruits

### Conclusion

Fruits and cladode of *O. dillenii* have shown remarkable effects against several diseases which could partly be confirmed by recent investigations and may be of special interest with respect to the rapidly increasing prevalence of diabetes type 2 in many parts of the world consequently. As reported above several of these secondary metabolites occur in *O. dillenii* and they may contribute a great deal to the antioxidant activity of a different solvent extract obtained from cladode and fruits

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