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PHARMACOGNOSTIC EVALUATION OF THREE DIFFERENT SPECIES OF *OPUNTIA*

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Abstract: **Pharmacognosy is the simple and reliable tool by which complete information of the curd drug can be obtained. In this context, authentication and quality assurance of medicinal plants, pharmacognostic, physicochemical, fluorescence analysis, extractive value and organoliptic characters were determined. Studies of three different spp. of Opuntia were carried out. The macroscopic evaluation revealed characters that are of diagnostic value and useful in authentication of the plant. The Physicochemical analysis reveals values for moisture content, organic solvent extractive, water extractive and total ash. Information obtained from these studies can be used as markers in the identification and standardization of these plants as an herbal remedy.**

Keywords: **Opuntia, physicochemical, fluorescence, fruit, cladode.**

1. INTRODUCTION

Plants are used as medicine to maintain human health from ages [1]. Plants are also major natural sources of medicinal compounds in current pharmacopoeias [2]. Indian Materia Medica includes about 2000 drugs of natural origin and most of them are derived from different traditional systems and folklore practices [3]. However, there are large numbers of plants which have not been mentioned in these reports, in spite of their usage in the traditional and folk medicinal systems. However a key obstacle, which has hindered the promotion in use of alternative medicines in the developed countries, is no evidence of documentation and absence of stringent quality control measures. There is a need for the record of all the research work carried out on traditional medicines in the form of documentation. With this drawback, it becomes extremely important to make surety about the standardization of the plant and parts of plant to be used as a medicine. For the process of standardization, we can use different techniques and methodology to achieve our goal in the stepwise manner e.g. pharmacognostic and phytochemical studies. These steps and processes are helpful in identification and standardization of the plant material. Correct characterization and quality assurance of starting material is an essential step to ensure reproducible quality of herbal medicine which will help us to justify its safety and efficacy [4-7]. For this purpose we have done pharmacognostic studies of three different spp. of *Opuntia. Opuntia* belongs the family Cactaceae is a xerophyte represented with about 200 – 300 species worldwide mainly grown in arid and semi-arid zones. Due to their remarkable genetic variability, the plant shows a high ecological adaptivity and therefore, encountered in places of virtually all climatic conditions North, Central and South America, the Mediterranean, North, Central and South Africa, the Middle East, Australia, and India [8,9]. Traditionally, cactus plants serve as the sources of fruits and vegetables, medicine and cosmetics, forage, building material and natural colors. However, their uses are still mainly restricted to the countries of origin [10-14]. *Opuntia* fruits also known as cactus pears or prickly pears are regionally consumed in the form of fruit and juice and also exported to the European market [15,16]. Recent investigations anticipate the use of fruit juice as the functional ingredient for the soft drink market and betalainic coloring foodstuff [17,18]. The reports also reveals the presence of natural cactus molecules have a high potential interest in human health and medicine [19,20].Hence there is no reports on this plant with regards to pharmacognosy, so present study were carried out.

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2. MATERIALS AND METHODS

Collection and identification of plant material

All the three different spp. namely *Opuntia Cochenillifera*, *Opuntia Ficus indica* and *Opuntia Elatior* were collected from Hyderabad Karnataka Region. 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 [21-23].

Physical evaluation

The ash value, extractive value, loss and drying value were performed according to the methods prescribed in Indian pharmacopeia (24) and WHO (25) guidelines of quality control methods for medicinal plants materials. Fluorescence analysis was carried out according to the methods of Chass and Pratt (26) and Kokoski (27).

3. RESULTS

Organoleptic studies

Opuntia Cohenillifera

The physical evaluation of drugs is an important parameter in detecting adulteration or important handling of drug. The following Soxhlet extracts i.e. petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts of *O. cohenillifera* yields were determined for both parts, cladode as well as fruit and their differential values are recorded in the table I.

The weight of cladode extracts are 4.5 (pet. ether), 1.8 (chloroform), 2.2 (ethyl acetate), 5.9 (methanol) and 4.2 (water) grams respectively. And the weight of fruit extracts are 2.2 (pet. ether), 0.8 (chloroform), 3.2 (ethyl acetate), 6.2 (methanol) and 5.6 (water) grams respectively. It is evident from these results, that the methanol solvent has higher extractive values in case of both cladode and fruits as compared to other extracts.

The crude extracts of cladode and fruit of *O. cohenillifera* have shown a wide range of colour. In case of cladode, the petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts shows dark green, dark green, coffee, chocolate and green colours respectively. However in case of fruit part, the petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts shows yellow green, coffee, brown, dark brown and red colours respectively.

Cladode extract of *O. cohenillifera* exhibits same taste of bitterness for all solvents. While the fruit extracts exhibit salty taste for petroleum ether and chloroform, sweet taste for methanol and water whereas ethyl acetate extract shows bitter taste.

Further the nature of these extracts varies from solvent to solvent. In case of cladode, petroleum ether and aqueous extracts are in dry powder form, chloroform and ethyl acetate extracts are waxy in nature whereas methanol extract show sticky nature. In case of fruit, petroleum ether, chloroform and ethyl acetate are waxy in nature whereas methanol and water extracts are sticky in nature. Similarly, the extracts were exhibiting variety of odour as shown in following table.

Table 1: Extractive values and organoleptic characters of *O. cohenillifera*

Solvents	Weight of the extract (g)		Colour of the extract		Taste of the extract		Nature of the extract		Odour of the extract	
	Cladode	Fruit	Cladode	Fruit	cladode	Fruit	Cladode	Fruit	Cladode	Fruit
Petroleum ether	4.5	2.2	Dark green	yellow green	Bitter	Salty	Dry powder	Waxy	Aromatic	Aromatic
Chloroform	1.8	0.8	Dark green	Coffee	Bitter	Salty	Waxy	Waxy	Aromatic	Sweet
Ethyl acetate	2.2	3.2	Coffee	Brown	Bitter	Bitter	Waxy	Waxy	Aromatic	Sweet
Methanol	5.9	6.2	Chocolate	Dark brown	Bitter	Sweet	Sticky	Sticky	Sweet	Sweet
Water	4.2	5.6	Green	Red	Bitter	Sweet	Powder	Sticky	Sweet	Sweet

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Opuntia ficus indica

The following Soxhlet extracts i.e. petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts of *O. ficus indica* yields were determined for both parts, cladode as well as fruit and their values are recorded in the table II

The weight of cladode extracts are 2.6 (pet. ether), 1.2 (chloroform), 1.6 (ethyl acetate), 4.6 (methanol) and 4.4 (water) grams respectively. And the weight of fruit extracts are 2.8 (pet. ether), 1.3 (chloroform), 2.2 (ethyl acetate), 4.2 (methanol) and 3.8 (water) grams respectively. It is evident from these results, that the methanol solvent has higher extractive values in case of both cladode and fruits as compared to other extracts.

The crude extracts of cladode and fruit of *O. ficus indica* have shown a wide range of colour. In case of cladode, the petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts shows light green, dark green, black, brown and green colours respectively. However, in case of fruit part, the petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts shows light brown, brown yellow, dark brown, red yellow and dark red colours respectively.

Cladode extract of *O. ficus indica* exhibits same taste of bitterness for all solvents. While the fruit extracts exhibit salty taste for petroleum ether, bitter for chloroform and sweet taste for methanol ethyl acetate and aqueous solvents.

Further the nature of these extracts varies from solvent to solvent. In case of cladode, petroleum ether, chloroform and aqueous extracts are in dry powder form. Ethyl acetate was sticky and methanol was waxy in nature. In case of fruit, petroleum ether and aqueous extracts are powder in nature, ethyl acetate and methanol extracts are waxy in nature whereas chloroform extract was having sticky nature. Similarly, the extracts were exhibiting variety of odour as shown in following table.

Table II: Extractive values and organoleptic characters of *O. ficus indica*

Solvents	Weight of the extract (g)		Colour of the extract		Taste of the extract		Nature of the extract		Odour of the extract	
	Cladode	Fruit	Cladode	Fruit	Cladode	Fruit	Cladode	Fruit	Cladode	Fruit
Petroleum ether	2.6	2.8	Light green	Light brown	Bitter	Salty	Dry powder	Dry powder	Aromatic	Aromatic
Chloroform	1.2	1.3	Dark green	Brown yellow	Bitter	Bitter	Powder	Sticky	Aromatic	Aromatic
Ethyl acetate	1.6	2.2	Black	Dark brown	Bitter	Sweet	Sticky	Waxy	Pungent	Pungent
Methanol	4.6	4.2	Brown	Red yellow	Bitter	Sweet	Waxy	Waxy	Pungent	Sweet
Water	4.4	3.8	Green	Dark red	Bitter	Sweet	powder	powder	odourless	odourless

Opuntia Elatior

The following Soxhlet extracts i.e. petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts of *O. elatior* yields were determined for both parts, cladode as well as fruit and their values are recorded in the table III

The weight of cladode extracts are 4.3 (pet. ether), 2.3 (chloroform), 1.8 (ethyl acetate), 4.5 (methanol) and 4.2 (water) grams respectively. And the weight of fruit extracts are 5.5 (pet. ether), 1.8 (chloroform), 1.2 (ethyl acetate), 8.4 (methanol) and 7.1 (water) grams respectively. It is evident from these results, that the methanol solvent has higher extractive values in case of both cladode and fruits as compared to other extracts.

The crude extracts of cladode and fruit of *O. elatior* have shown a wide range of colour. In case of cladode, the petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts shows light green, dark green, brown, chocolate and green colours respectively. However in case of fruit part, the petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts shows yellow green, brown, dark brown, chocolate red and red colours respectively.

Cladode extract of *O. elatior* exhibits same taste of bitterness for all solvents. While the fruit extracts exhibit sweet taste for ethyl acetate, methanol and aqueous solvents. However petroleum ether and chloroform extracts were tasteless.

Further the nature of these extracts varies from solvent to solvent. In case of cladode, petroleum ether and aqueous extracts are in powder form. Ethyl acetate, chloroform and methanol were sticky in nature. In case of fruit, petroleum ether was waxy in nature, chloroform, ethyl acetate and methanol extracts are sticky in nature whereas aqueous extract was powder in nature. Similarly, the extracts were exhibiting variety of odour as shown in following table.

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Table III: Extractive values and organoleptic characters of *O. elatior*

Solvents	Weight of the extract		Colour of the extract		Taste of the extract		Nature of the extract		Odour of the extract	
	Cladode	Fruit	Cladode	Fruit	Cladode	Fruit	Cladode	Fruit	Cladode	Fruit
Petroleum ether	4.3	5.5	Light green	Yellow green	Bitter	Tasteless	Powder	Waxy	Aromatic	Aromatic
Chloroform	2.3	1.8	Dark green	Brown	Bitter	Tasteless	Sticky	Sticky	Aromatic	Aromatic
Ethyl acetate	1.8	1.2	Brown	Dark brown	Bitter	Sweet	Sticky	Sticky	Aromatic	Sweet
Methanol	4.5	8.4	Chocolate	Chocolate red	Bitter	Sweet	Sticky	Sticky	Sweet	Sweet
Water	4.2	7.1	Green	Red	Bitter	Sweet	powder	Powder	odourless	Odourless

Total ash, water soluble and acid insoluble values

The ash values, acid insoluble, water insoluble, alcohol insoluble, fiber content and moisture content values in cladode and fruit of all three species of *opuntia* were determined and the results are shown in table IV

Total ash value, in case of cladode, was significantly high in species *O. elatior* (4.19g) followed by *O. ficus indica* (2.458g) and *O. cohenillifera* (1.745g). However in case of fruit, total ash value was high in *O. cochenillifera* (3.992g) followed by *O. ficus indica* (2.123g) and *O. elatior* (1.75g).

Fibre content, in case of cladode, was considerably high in *O. elatior* (0.95g) followed by *O. ficus indica* (0.58g) and *O. cohenillifera* (0.45g). However in case of fruit, it was high in *O. elatior* (0.37g) followed by *O. ficus indica* (0.32g) and *O. cohenillifera* (0.22g).

The moisture content, in case of cladode, was noticeably high in *O. ficus indica* (0.66g) followed by *O. elatior* (0.58g) and *O. cohenillifera* (0.53g). But in case of fruit, it was high in species *O. ficus indica* (0.32g) followed by *O. cochenillifera* (0.31g) and *O. elatior* (0.28g).

Table IV: Physicochemical parameters of all three *opuntia* species

Type of Species	Part Used	Total ash for 10g powder	Acid insoluble ash content (500mg)	Water insoluble ash content (500mg)	Alcohol insoluble ash content (500mg)	Fibre content (500mg)	Moisture content (500mg)
O. cochenillifera	Cladode	1.745	54	92	72	45	253.4
	Fruit	3.992	36	164	12	22	161.8
O. ficus indica	Cladode	2.458	52	92	66	58	226.2
	Fruit	2.123	74	88	23	32	136.6
O. elatior	Cladode	4.190	40	136	98	95	218.4
	Fruit	1.75	66	90	45	37	123.7

Fluorescence analysis

Fluorescence analysis is the tool to determine the kind of nature of the drug. The fluorescence analysis of the plant powder was done by treating it with various chemical reagents and separate observations were made under normal light and UV light. The changes in colour due to treatment of different solvents with powder of three different species of *Opuntia* was recorded and presented in the tables V, VI and VII

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Fluorescence analysis *O. cochenillifera*

Cladode dried powder with petroleum ether, chloroform, ethyl acetate, methanol, ether, acetone, ethanol, benzene, concentrated HCl, concentrated H_2SO_4 appears as light green, dark green, green, dark green, green, light green, yellow green, yellow, brown, black under normal light and light green, yellow green, yellow green, yellow green, light green, light green, light green, transparent, black, black under UV light respectively.

Similarly, fruit dried powder with petroleum ether, chloroform, ethyl acetate, methanol, ether, acetone, ethanol, benzene, concentrated HCl, concentrated H_2SO_4 appears as dark brown, red, dark red, light red, brown, pink, red, black, black under normal light and blue, dark red, light red, brown, pink, blue, dark blue, blue, black, black under UV light respectively

Table V: Fluorescence analysis of *O. cochenillifera*

Solvent treatment with powder	Visible light		UV 365	
	Cladode	Fruit	Cladode	Fruit
Only Powder	Light green	Brown	White	White
Petroleum ether	Light green	Dark brown	Light green	Blue
Chloroform	Dark Green	Red	Yellow green	Dark red
Ethyl acetate	Green	Dark red	Yellow green	Light red
Methanol	Dark Green	Dark red	Yellow green	Brown
Ether	Green	Light red	Light green	Pink
Acetone	Light green	Brown	Light green	Blue
Ethanol	Yellow green	Pink	Light green	Dark Blue
Benzene	Yellow	Red	Transparent	Blue
Conc. HCl	Brown	Black	Black	Black
Conc. H_2SO_4	Black	Black	Black	Black

Fluorescence analysis *O. ficus indica*

Cladode dried powder with petroleum ether, chloroform, ethyl acetate, methanol, ether, acetone, ethanol, benzene, concentrated HCl, concentrated H_2SO_4 appears as green, dark green, green, dark green, yellow green, light yellow, yellow green, black, black under normal light and transparent, transparent, transparent, yellow green, transparent, white, light green, green, black, black under UV light respectively.

Similarly fruit dried powder with petroleum ether, chloroform, ethyl acetate, methanol, ether, acetone, ethanol, benzene, concentrated HCl, concentrated H_2SO_4 appears as dark orange, light yellow, dark yellow, light yellow,yellow, yellow orange, grey yellow, light yellow, black, black under normal light and light blue, blue, dark yellow, blue, white, blue, light yellow, yellow, black, black under UV light respectively.

Table VI: Fluorescence analysis of *O. ficus indica*

Solvent treatment with powder	Visible light		UV 365	
	Cladode	Fruit	Cladode	Fruit
Only Powder	Green	Light Orange	White	White
Petroleum ether	Green	Dark orange	Transparent	Light blue
Chloroform	Dark green	Light yellow	Transparent	Blue
Ethyl acetate	Green	Dark yellow	Transparent	Dark yellow
Methanol	Dark green	Light yellow	Yellow green	Blue
Ether	Yellow green	Yellow	Transparent	White
Acetone	Light yellow	Yellow orange	White	Blue

Ethanol	Yellow green	Gray yellow	Light green	Light yellow
Benzene	Yellow green	Light Yellow	Green	Yellow
Conc HCl	Black	Black	Black	Black
Conc H_2SO_4	Black	Black	Black	Black

Fluorescence analysis *O. elatior*

Cladode dried powder with petroleum ether, chloroform, ethyl acetate, methanol, ether, acetone, ethanol, benzene, concentrated HCl, concentrated H_2SO_4 appears as dark green, yellow green, light yellow, yellow green, green, dark green, green, light yellow, black, black under normal light and dark green, yellow green, yellow green, yellow green, light green, light green, light green, transparent, black, black under UV light respectively.

Similarly, fruit dried powder with petroleum ether, chloroform, ethyl acetate, methanol, ether, acetone, ethanol, benzene, concentrated HCl, concentrated H_2SO_4 appears as brown, dark brown, red, dark red, dark red, light red, brown, red, black, black under normal light and orange, red, dark red, blue, dark blue, dark blue, light blue, orange red, black, black under UV light respectively.

Table VII: Fluorescence analysis of *O. elatior*

Solvent treatment with powder	Visible light		UV 365	
	Cladode	Fruit	Cladode	Fruit
Only Powder	Light green	Light red	white	White
Petroleum ether	Dark green	Brown	Dark green	Orange
Chloroform	Yellow green	Dark brown	Yellow green	Red
Ethyl acetate	Light Yellow	Red	Yellow green	Dark red
Methanol	Yellow green	Dark red	Yellow green	Blue
Ether	Green	Dark red	Light green	Dark blue
Acetone	Dark green	Light red	Light green	Dark blue
Ethanol	Green	Brown	Light green	Light blue
Benzene	Light yellow	Red	Transparent	Orange red
Conc HCl	Black	Black	Black	Black
Conc H_2SO_4	Black	Black	Black	Black

4. DISCUSSION

The total ash is particularly important in the evaluation of purity of the drugs, i.e. the presence or absence of foreign organic matter such as metallic salts and/or silica [28]. In the present study, the total ash content found was high in *O. elatior* (41.9%) and *O. cochenillifera* (29%) in cladode and fruit respectively compared to remaining two species. Ash content of a drug indicates the presence of various impurities like carbonates, oxalates and silicates present along with the drug. The water soluble ash is used to determine the amount of inorganic compounds present in herbal drugs. Acid insoluble ash gives an idea about the amount of silica present and indicates contamination with earthy material [29].

Estimation of moisture content is significant for the material which absorbs moisture easily or deteriorates quickly in the presence of water. Thus the moisture content is a parameter for checking the purity of the drug. In the present study, moisture content found was high in *O. cochenillifera* (51%) cladode compared to other two species. While Hemil Patel *et.al* [30] evaluated and obtained 74.16% moisture content in fruit of *O. elatior* Mill.

Fluorescence analysis

The fluorescence study of powder under UV light is useful in establishing the purity of the drug. It can be checked by observing the change in color of powder under UV light after treating the powder with different chemicals. Many

phytocompounds will fluoresce when suitably illuminated. The fluorescence color is specific for each compound. The non fluorescent compound may be fluorescent if it is mixed with impurities that are fluorescent. The fluorescent method is effectively sensitive and enables the precise and accurate determination of analyze over an adequate concentration range without several time consuming dilution steps earlier to analysis of pharmaceutical samples [31].

In the present study, the powdered cladodes and fruits of three *Opuntia* spp. emitted wide range of colours under daylight and UV light. Fluorescence analysis of different plant parts in powder form provides a clue that if powder is in adulteration, so can be used as a diagnostic tool for testing the adulteration. Presence or absence of certain main compounds in an extract is determined by colour reactions of the compounds with specific chemicals which act as dyes. This procedure is requirement before going for detailed phytochemical investigation. Therefore, some crude drugs are often assessed qualitatively in this way and it is a key parameter of pharmacognostical evaluation [32].

5. CONCLUSION

Opuntia spp. are widely grown in India for its various purposes. The pharmacognostic investigation on physicochemical characteristics and fluorescence analysis shows that authentic botanical of this crude drug will prevent adulteration substitution and has a crucial role in standardization of crude drug. By above all these parameters we can build up a suitable plant profile which paves way for further studies on the plant for the presence of active compounds and their biological activity. Therefore development of authentic analytic methods including pharmacogonostical physicochemical parameter is established to ensure the safety, efficacy and purity of these important plants.

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