PRELIMINARY PHYTOCHEMICAL SCREENING AND THIN LAYER CHROMATOGRAPHY OF DRAGON FRUIT FLESH, PEEL AND SPINES

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ABSTRACT: Dragon fruit is an exotic fruit belongs to the family Cactaceae. Earlier reports said that to have all the activities such as anti-inflammatory, anti-oxidant, anti-diabetic, antimicrobial, anti-cancer, improve eyesight, source of phenolic compounds. In present study we have taken Indian cultivated fruit (white flesh variety) and its Spines, Peel and flesh (alcohol extract) are subjected to Preliminary phytochemical screening and thin layer chromatography studies for flavonoids. The present study shows that alkaloids, saponin glycosides, flavonoids, steroids and Triterpenoids are present in all parts of the fruit. Based on their therapeutic claims in literature and reporting from Australian variety, we checked for the presence of Flavonoids and further identified by thin layer chromatography. Flavonoids were present in all 3 parts of the fruit, so all 3 parts can be used as a source of flavonoids. Further studies to be carried out for isolation and identification of flavonoids compounds. The fruit has potential to be recommended as nutraceutical as a source of antioxidants.

Keywords: Dragon fruit, Peel, Spines, Flesh, Preliminary Phytochemical Screening, Thin Layer Chromatography Studies, Flavonoids.

I. INTRODUCTION

Hylocereus undatus belongings to the family of Cactaceae. This fruit survived in the dry tropical climate and can withstand temperature as high as 40^{o}C (Ortizhernández and Carrillo-salazar, 2012) It is well known under the name of "dragon fruit" or "pitaya". There are three different species of Pitaya fruit which include *Hylocereus undatus* or white pulp with red fruit extract Pitaya fruit. The fruit of *Hylocereus undatus* is large in size, oval shape, weighing about 280-5060 grams, 33-36 cm diameter and 14-16 cm long. The fruit has delicate and sweet flesh with intense white colour of the flesh and red-purple colour of seeds extract. The fruits often consumed fresh or made into juices, cordial, jams and ice cream, sherbets, yogurt, candy and pastries. It has a lot of tiny black seeds, which are rich in essential fatty acids. The *Hylocereus undatus* fruit are rich in fibre, vitamins, calcium, phosphorus, magnesium, phytochemicals and antioxidants. Betanin, phyllotactic, hylocerenin, and betacyanin with 5– *O*-glycosides or 6-*O*-glycosides have been discovered in many species of the Cactaceae family. Phytochemical screening of the white dragon fruit showed the presence of triterpenoid, alkaloid, flavonoid and saponins. *Hylocereus undatus* extract was found to decrease the duration of catalepsy significantly. *Hylocereus undatus* fruit is also known to possess medicinal and pharmaceutical properties that could prevent diabetes, cancer and neutralizes toxins in body. It is even helpful in reducing blood sugar levels in Type 2 diabetic patients.

There were several studies mentioned to the processing and preservation of dragon fruit (*Hylocereus undatus).* The proximate composition of dragon fruit (*Hylocereus undatus*) was analysed to develop dragon fruit jelly. Studies on preparation and storage of jelly from dragon fruit (*Hylocereus undatus*) was examined. Quality of pitaya fruit (*Hylocereus undatus*) as influenced by storage temperature and packaging was studied. Production of a novel fruit-yoghurt using dragon fruit (*Hylocereus undatus* L.). Effects of blanching and drying on fibre rich powder from pitaya (*Hylocereus undatus*) peel was examined. The albedo powder of dragon fruit could be used for food colouring. Research aimed to investigate different factors influencing on the production of dragon fruit wine. Dragon fruit (*Hylocereus undatus* is an interested agricultural product since its antioxidative activity from its fruit pigments, betalains group. In order diversify different products from dragon fruit (*Hylocereus undatus),* objective of this search focused on the effect of pH, sugar supplementation, temperature and time of cooking to sensory score of dragon fruit nectar; changes of total soluble solid, acidity and microbial load in dragon fruit nectar during storage.

II. MATERIALS AND METHODS

Plant material

The flesh, peel and pulp of *Hylocereus undatus* were collected from the local market of Nalgonda, Telangana, India. The plant material was washed thoroughly with distilled water to remove surface contaminants and separated, and each part of the fruit i.e., peel, spines, pulp macerated and juice was subjected to shade dried for 20 days. Each sample of the material was ground separately to fine powder using electrical blender and stored in air tight container at ambient temperature.

III. EXTRACTION PROCESS

In this method of extraction maceration involved soaking plant material (coarse or powdered) in a stoppered container with a suitable solvent i.e., Ethanol and allowed to stand it at room temperature for period of minimum 3 days with frequent agitation, the processed intended to soften and break the plant's cell wall to release the soluble phytochemicals. After 3 days, the mixture is pressed or strained by filtration. In this conventional method, heat is transferred through conduction and convection and the choice of solvents will determine the type of compound extracted from samples. Infusion and decoction use the same principle as Maceration; both are soaked in cold or boiled water. However, the maceration period for infusion is shorter and the sample boiled in specified volume of water (i.e., 1:4 or 1:16) for a defined time for decoction. To ensure optimal extraction the alcohol is changed for every two days. By changing the alcohol regularly, we prevented the build-up of impurities and maximize the extraction of flavours and compounds from dragon fruit parts. The maceration process with alcohol helps breakdown the cell walls of dragon fruit pulp, peel and spines releasing their aromatic components into liquid. The repeated alcohol changes help maintain a clean and efficient extraction resulting in high quality macerated product. The alcohol used in the maceration procedure acts as a solvent effectively drawing out the desired flavours and compounds from the dragon fruit pulp, peel and spines. Regularly changing the alcohol allows for continuous extraction ensuring that all the available flavours are captured from the dragon fruit. The maceration procedure with alcohol and regular alcohol changes is a common technique employed in the extraction of the botanical flavours from various plant materials including dragon fruit pulp, peel and spines. Seal the container with aluminium foils stored it in a cool dark place to allow the flavours to infuse. .Periodically gently stir or shake the container to distribute flavours and ensure all ingredients are exposed to liquid.

Preliminary phytochemical analysis:

The alcoholic extract of *Hylocereus undatus* Spines, Peel, Flesh was subjected to preliminary phytochemical analysis.

Tests performed for the presence of phytoconstituents

A) Test for alkaloids:

0.5 g of extract was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Draggendorff's reagent to the other.

1. Dragendroff's test:

To1 ml of each of the sample solution taken in a test tube few drops of Dragendroff's reagent (potassium bismuth iodide solution) was added. A reddish brown precipitate was observed indicating the presence of alkaloids.

2. Mayer's test:

To 1ml of each of sample solution few drops of Mayer's reagent (potassium mercuric chloride solution) was added. A creamish white precipitate was formed indicating the presence of alkaloids.

3. Wagner's test:

To few ml of each of the sample solution, Wagner's reagent (iodine in potassium iodide) was added, which resulted in the formation of reddish-brown precipitate indicating in the presence of Alkaloids.

4. Hager's test:

To 1ml of each of the sample few drops of Hager's reagent(picric acid) was added. Yellow precipitate was formed reacting positively for alkaloids.

B) Test for Sterols and Triterpenoids:

1. Libermann-Buchard test:

When samples were treated with few drops of acetic anhydride, boiled and few drops of concentrated sulphuric acid from the sides of the test tube were added, shows a brown ring at the junction of two layers and the upper layer turns green which shows the presence of steroids.

2. Salkowski test:

Few drops of concentrated sulphuric acid were added to the test samples in chloroform, a red colour appears at the lower layer indicates the presence of sterols.

C) Test for flavonoids:

Three methods were used to test flavonoids. Dilute ammonia (5 ml) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1 ml) was added. A yellow colouration on standing indicates the presence of flavonoids. Second, a few drops of 1% aluminium solution were added to a portion of the filtrate. Third, a portion of the extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution.

1. Alkaline reagent test:

When sodium hydroxide solution was added to the test samples formation of intense yellow colour, which turns to colour less on addition of few drops of dilute acid indicates the presence of flavonoids.

D) Test for saponins:

To 0.5 gm of extract, 5 ml of distilled water was added in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

1.Foam test:

5ml of extract was shaken vigorously to obtain a stable persistent froth. The froth was then mixed with 3 drops of olive oil and observed for the formation of an emulsion, which indicates the presence of saponins.

THIN LAYER CHROMATOGRAPHY OF DRAGON FRUIT FLESH, PEEL, SPINES:

Thin layer chromatography (TLC) is a chromatography technique used to separate mixtures of chemical compounds. It is the most basic method of confirming the presence of a phytochemical compound.

TLC for flavonoids:

Adsorbent : Pre-coated Silica Gel 60F 254
Solvent system : 1. Toluene: Acetone: Formic acid (3:1:1)
2. Toluene: Ethyl acetate: Formic acid (3.5: 1:1)
3. Toluene: Acetic acid (4:2)
4. Chloroform: ethyl acetate (6:4)
Spraying reagent : 2% AlCl3
Visualization : UV Chamber

Thin layer chromatography was performed as per standard procedure.

Thin layer chromatographic analysis (TLC)

The Alcoholic extract which contains major constituents was subjected to thin layer chromatography in order to detect and confirm phytoconstituents like Flavonoids. The TLC for flavonoids with different solvent systems showed different spots in different colours under UV light in three different wavelengths with Rf values which are tabulated in table and figures. After derivatization with aluminium chloride the quenching of the fluorescent spots were observed.

IV. RESULTS

Preliminary phytochemical screening:

The FLESH, PEEL AND SPINES of *H.undatus* was subjected to successive maceration using absolute alcohol. The obtained extracts were subjected to preliminary phytochemical screening according to the standard procedures mentioned **.** Findings were tables.

Qualitative Preliminary Phytochemical screening of SPINES of *H.undatus*

Chemical constituents	**Alcohol extract**
Alkaloids:	PRESENT
Dragendroff's reagent	-
Mayer's reagent	+
Wagner's reagent	-
Hager's reagent	+

Steroids and Triterpenoids	PRESENT
Libermann-Buchard test	+
Salkowski test	+
Flavonoids	PRESENT
Alkaline reagent test	+
Saponin glycosides	PRESENT
Froth formation test	+

Qualitative Preliminary Phytochemical screening of PEEL of *H.undatus*

Chemical constituents	**Alcohol extract**
Alkaloids:	PRESENT
Dragendroff's reagent	-
Mayer's reagent	+
Wagner's reagent	-
Hager's reagent	+
Steroids and Triterpenoids	PRESENT
Libermann-Buchard test	+
Salkowski test	+
Flavonoids	PRESENT
Alkaline reagent test	+
Saponin glycosides	PRESENT
Froth formation test	+

Qualitative Preliminary Phytochemical screening of FLESH of *H.undatus*

Chemical constituents	**Alcohol extract**
Alkaloids:	PRESENT
Dragendroff's reagent	+
Mayer's reagent	+
Wagner's reagent	+
Hager's reagent	+
Steroids and Triterpenoids	PRESENT
Libermann-Buchard test	+
Salkowski test	+
	+
Flavonoids	PRESENT
Alkaline reagent test	+
Saponin glycosides	PRESENT
Froth formation test	+

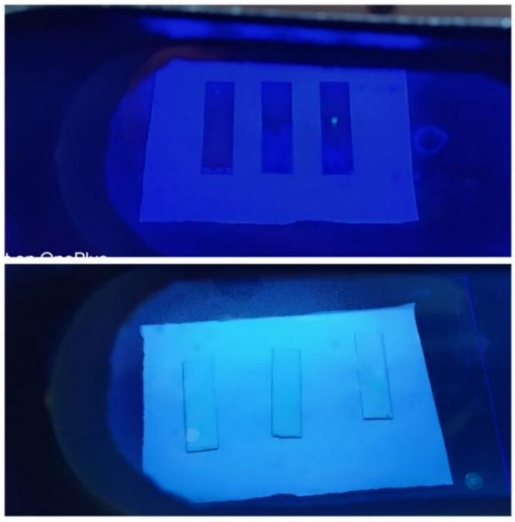
The Preliminary Phytochemical studies revealed that the *Hylocereus undatus* contained chemical constituents like Alkaloids, Steroids and Triterpenoids, Flavonoids and Saponin glycosides.

Thin layer chromatographic analysis (TLC)

The Alcoholic extract which contains major constituents was subjected to thin layer chromatography in order to detect and confirm phytoconstituents like Flavonoids. The TLC for flavonoids with different solvent systems showed different spots in different colours under UV light in three different wavelengths with Rf values which are tabulated in table and figures. After derivatization with aluminium chloride the quenching of the fluorescent spots were observed.

Thin Layer Chromatography of SPINES of *H.undatus*

SOLVENT SYSTEM	**LONG WAVELENGTH (365nm)**		**SHORT WAVE LENGTH (256 nm)**		**DAY LIGHT**	
Toluene: Ethyl acetate: Formic acid (3:1:1)	**Spot colour**	R_f	**Spot colour**	R_f	**Spot colour**	R_f
	Green Light green Yellow	0.25 0.56 0.36	Green Yellow	0.25 0.36	Yellow Red	0.36
Toluene: Acetic acid (4:2)	Green Yellow Light green	0.35 0.51 0.62	Green Yellow	0.35 0.51	Yellow	0.51
Chloroform: Ethyl acetate (6:4)	Green Yellow Light Green	0.30 0.48 0.74	Green Yellow	0.30 0.48	Yellow	0.48

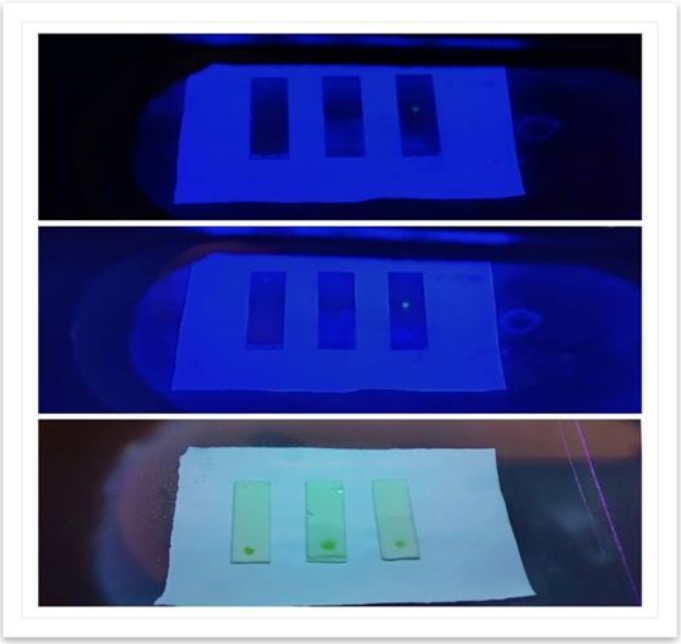


LONG WAVELENGTH, SHORT WAVELENGTH OF SPINES

Thin Layer Chromatography of PEEL of *H.undatus*

SOLVENT SYSTEM	**LONG WAVELENGTH (365nm)**		**SHORT WAVE LENGTH (256 nm)**		**DAY LIGHT**	
Toluene: Ethyl	**Spot colour**	R_f	**Spot colour**	R_f	**Spot colour**	R_f

acetate: Formic acid (3:1:1)	Green Light green Brown	0.58 0.2 0.75	Green Yellow	0.58	Yellow Brown Green	 0.75 0.58
Toluene: Acetic acid (4:2)	Light Green Green Brown	0.25 0.5 0.76	Green Yellow	0.5	Green Yellow	0.5
Chloroform: Ethyl acetate (6:4)	Green Brown Light Green	0.55 0.9 0.32	Green Yellow	0.55	Green Yellow	0.55

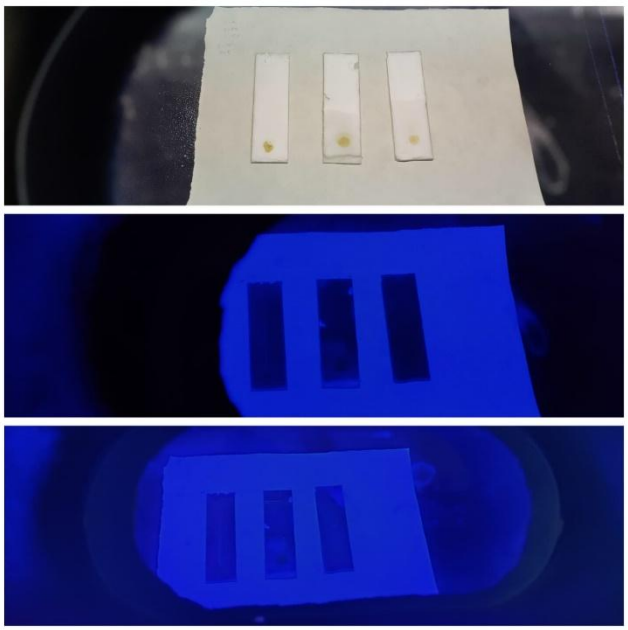


LONG WAVELENGTH, SHORT WAVELENGTH, DAY LIGHT OF PEEL

Thin Layer Chromatography of FLESH of *H.undatus*

SOLVENT SYSTEM	**LONG WAVELENGTH (365nm)**		**SHORT WAVE LENGTH (256 nm)**		**DAY LIGHT**	
Toluene: Ethyl acetate: Formic acid (3:1:1)	**Spot colour**	R_f	**Spot colour**	R_f	**Spot colour**	R_f
	Green Brown Yellow	0.54 0.41 0.21	Green Yellow	0.54 0.21	Yellow Green Brown	0.21 0.54

Toluene: Acetic acid (4:2)	Green Yellow Brown	 0.42 0.28	Green Yellow	0.18 0.42	Green Yellow Brown	0.18 0.42
Chloroform: Ethyl acetate (6:4)	Green Yellow Brown	 0.24 0.72	Green Yellow	0.72 0.24	Green Yellow Brown	0.72 0.24



DAY LIGHT, SHORT WAVELENGTH, LONG WAVELENGTH OF PULP

V. DISCUSSION

The preliminary phytochemical screening is used to know the presence of various chemical constituents present in the *H.undatus* extract. Then the extract was subjected to the preliminary tests in which the aqueous extract had shown the presence of various constituents like flavonoids and saponins**.**

Hence the absolute alcohol was selected as the suitable solvent for the extraction. The information obtained from preliminary phytochemical screening will be useful in finding out the Genuity of the *H.undatus*. The presence of Flavonoids can be confirmed by Thin Layer Chromatography(TLC). Chromatography's are strongly recommended for the purpose of quality control of herbal medicines, since they might represent appropriately the chemical integrities of the herbal medicines and therefore be used for authentication and identification of the herbal products. The R_f values calculated from the TLC using adsorbents and different solvent systems helps to identify various chemical constituents.

The TLC analysis of Spines, Peel, Pulp of *H.undatus* showed spots for flavonoids in three different solvent systems and the R_f values were obtained and tabulated.

The TLC analysis of flavonoids showed fluorescent quenching after spraying with the aluminium chloride. The fluorescent quenching was due to complex formation of flavonoids with Al^{3+}. The colours of the fluorescing zones can depend on the concentration of the aluminium chloride solution.

VI. CONCLUSION

Due to its nutritional and medicinal properties the dragon fruit brings numerous benefits to human health mostly for the control and management of the oxidative stress. All the different types of Pitaya (i.e., spines, peels and pulp) contain bioactive compounds involved in a wide range of beneficial biological activities including antioxidants, antimicrobial, anti-cancer, anti-inflammatory capacities these include betalains, flavonoids, polyphenols, terpenoid and steroids, saponins,

alkaloids, tannins and carotenoids, which has been proven as effective, healthier, safer and sustainable alternatives to synthetic drugs for the treatment and Prevention of many diseases such as diabetes, cancer, obesity, hyperlipidaemia and pathogenic agents such as virus bacteria and fungi. Besides the pharmaceutical value of its compound the Pitaya is also a national suit of colours with potential uses in the food and the cosmetic industries.

The preliminary phytochemical tests and thin-layer chromatography (TLC) analysis of flavonoids in *H.undatus* pulp, peel, and spines revealed the presence of various bioactive compounds. The results suggest that Dragon fruit, commonly known for its vibrant colour and unique appearance, possesses a rich source of flavonoids.

The preliminary phytochemical tests indicated the presence of flavonoids, which are known for their antioxidant, anti-inflammatory, and anti-cancer properties. The TLC analysis further confirmed the presence of multiple flavonoid compounds in the Dragon fruit samples, as evidenced by the distinct bands observed on the chromatogram.

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