ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 15, Issue, 04, pp.4644-4651, April, 2024

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

PHARMACOPEIAL STANDARD DEVELOPMENT, PHARMACOGNOSY, HPTLC. FINGERPRINTING AND PHYSICOCHEMICAL RESEARCH STUDIES OF *BENINCA HISPIDA* (THUNB.)

Pawan Kumar Sagar*[1], N. Z. Ahmed[2], R.P.Meena[3] and S. Kashyap[4]

[1 &4]Drug Standardization Research Institute, (Under CCRUM, Ministry of AYUSH., Govt. of India), PCIM&H Campus, IInd Floor, Kamla Nehru Nagar, Ghaziabad-201002, U.P., India
[2,3]Central Council of Research in Unani Medicine, (Ministry of AYUSH., Govt. of India), P.NO. - 61-65, Institutional Area, Opp.-D, Block, Jankpuri New Delh-110058, India

DOI: http://dx.doi.org/10.24327/ijrsr.20241504.0869

ARTICLE INFO

Article History:

Received 27th February, 2023
Received in revised form 16th March, 2023
Accepted 16th April, 2024
Published online 28th April, 2024

Keywords:

(*Beninca hispida* (Thunb.), therapeutic disorders, Development of Pharmacopeial Standards, physicochemical screening, QC, QA.

ABSTRACT

(*Beninca hispida* (Thunb.) - Petha is a very important processed, dynamic commercial product found of North India. It is processed largely in North India- Uttar Pradesh, Uttarakhand plan area and Bihar States from wax gourd or ash gourd. Petha sweet is having geographical indication (GI) tag from Agra, U.P. State in India. BH. used as amazing therapeutic potentials as a Antioxidant, Antidiabetic, Hypoglycemic and Hypolipidemic, Anti-inflammatory, Alzheimer's disease, Anti-compulsive effect, Allergic inflammation, Antibiotic, Anti-microbial, Anti-Ulcer, Anti-Acidic, Anti-asthmatic, Cytotoxic and Anticancer, Anti-obesity, Antidepressant and Anxiolytic, Analgesic and Antipyretic, Antihypertensive, Anti ageing of Skin, Gastro protective from since ancient time. Three fresh samples of BH. taken into these studies from North India Region U.P., U.K. and Bihar State which 3 samples were freshly collected P1,P2 and P3. and developed from applied SOP. on the basis of authenticated classical texts and literature of standard methods. The quality control & quality assurance studies were conducted in accordance to the WHO., AOAC., IPC. and UPC., approved guidelines. The physicochemical data showed that the drug samples contain, Average values as Foreign Matter, w/w,(%)-Nil, LOD/ moisture (3.85%), Total Ash, w/w,(%)- (6.21%), Acid In-soluble, w/w,(%)- (0.32%), Alcohol and Water Soluble Extractive Matter, w/v, (%)- (2.50%) & (5.56%), pH(1% solution) (5.92), pH(10% solution) (5.35), and various bioactive phytochemical screening examined were assessed in BH., HPTLC. studies of chloroform and alcohol extracts of different samples P1.P2 and P3 of Petha BH. Classical formulation showed various spots at examine under 254nm and under 366nm (UV. region), found no major spots is observed, under exposed to Vinyl - Sulfuric acid reagent. derivatized obtained with equate, best separation using selected suitable solvent system of mobile phase. The quality control studies results revealed the absence of hazardous and toxic contamination from the drug samples of BH. Moreover the obtained research studies data and comparative screening will provide the referential supportive information in the development of Pharmacopeial standard monographs, identification of classical formulation, reinvestigation, quality assurance and pharmaco- vigilance and validation of the Quality standard of BH. classical sweet food providing the quality food used as a medicine to Health & Wellness of needful public mass of the world.

INTRODUCTION

Food and food products are being used as medicines over centuries worldwide. Many species from the family Cucurbitaceae have been used as medicaments in various diseases in Ayurveda and ancient Chinese medicine. This family is also known as the gourd family. It provides approximately 5 to 6% of the total vegetables in the world. To date, 825 species from under 118 genera have been reported growing in temperate regions of the world. (Ghebretinsae *et al.*, 2007) It should be mentioned that the Cucurbit species can grow in diverse climatic conditions, including arid deserts, tropical, subtropical, and temperate regions. These various types of species are included in food systems and Indian

Corresponding author:* **Pawan Kumar Sagar
Drug Standardization Research Institute, (Under CCRUM, Ministry of AYUSH., Govt. of India), PCIM&H Campus, IInd Floor, Kamla Nehru Nagar, Ghaziabad-201002, U.P., India

traditional medicines. Generally, the gourd family vegetables provide vitamins, essential minerals, antioxidants, and soluble fibers. (Palamthodi *et al*.,2014)). It is also observed that about 80% of the world population is using medicinal plants primarily in the developing countries for treating different diseases, due to their safety, efficacy, cultural acceptability and lesser side effect. It is important for herbal formulations to get the quality assurance by the conventional system of medicine, so that they can be *Benincasa hispida* (Thunb.) Cogn. (synonym: *Benincasa cerifera* Savi) (Cucurbitaceae) especially in Asian countries is considered as one of the famous crops under the Cucurbitaceae family that grows mainly for its fruits and well renowned for its nutritional and medicinal properties.(Purohit *et al*.,2019) Scientific reports suggest that *B. hispida* possesses many important nutritious substances, including vitamins, natural sugars, amino acids, organic acids, and mineral elements.(Purohit *et al*.,2019; Zaini *et al*., 2011; Andrias et al.,2019). It also contains smaller amounts of iron, magnesium, phosphorus, copper, and manganese, as well as various other B vitamins. Still, these amounts typically don't exceed 3% of the nutrients' DVs. In addition to vitamin C, ash gourd is a good source of flavonoids and carotenes, two antioxidants believed to help protect your body against cell damage and certain conditions like type 2 diabetes and heart disease.(Hadi *et al*.,2022; Boris *et al*., 2019) Currently, The subject of standardization of herbal drugs is massively wide and deep. There are many seemingly contradictory theories on the subject of herbal medicines and its relationship with human physiology and mental function. (Yadav *et al*.,2011; Sagar *et al*., 2022) The quality assurance and quality control of herbal crude drugs and formulated products are important in justifying their acceptability in modern system of medicine. Hence it is required to conduct the research on drugs standardization and product validation to provide effective, curable and safe drugs to the needy mass suffering from various ailments.(Sagar *et al*., 2023 a &b; 2022; 2020) All medicines, either synthetic or plant origin, have to fulfill the basic requirements of safety and efficacy. (EMEA, 2005; Anonymous, 2002)

Fresh Fruit of Petha, *Beninca Sahispida* (Thunb.) and Processed Candy of Ash gourd, *Beninca Sahispida* (Thunb.) clearly shown in Fig.1.a. and Fig.1b. respectively.

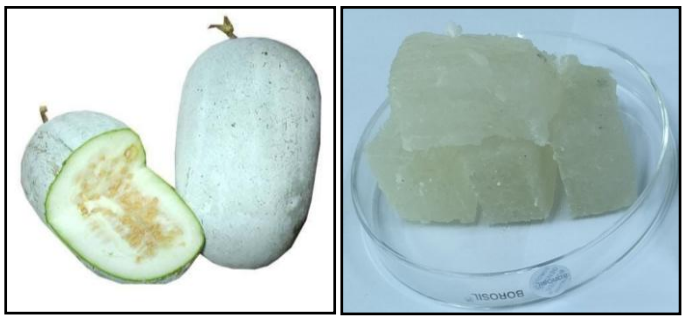


Fig.1a. Fig.1b.

Fig.1a. Fruit of Petha, *Beninca hispida* (Thunb.) and **Fig.1b.** Processed Candy of Ash gourd, *Beninca hispida* (Thunb.)

Pharmacological Activities

In-vitro & In-vivo Pharmacological Activities has investigated & reported in Petha - Ash Gourd (*Beninca hispida* (Thunb.)) as a Antioxidant, Antidiabetic, Hypoglycemic and Hypolipidemic, Anti-inflammatory, Alzheimer's disease, Anti-compulsive effect, Allergic inflammation, Antibiotic, General health prevention. (Gupta *et al*., 2019) Anti-microbial, Anti-Ulcer, Anti-Acidic,Anti-asthmatic, Diuretic, intestinal warm killer, act as blood coagulant, treat constipation. (Kalyani *et al*., 2020); Cytotoxic and Anticancer Effects, Antiobesity and Lipid-Lowering Effect, Antidepressant and Anxiolytic Effects, Analgesic and Antipyretic Effects, Antihypertensive Effect, Antiageing of Skin, Gastroprotective Effect. (Islam *et al*., 2021).

Regional & Different Nomenclature

Classical Names and Other Language Names: Urdu - Petha, Bengali - Chalkurma, Hindi- Petha or Kondha, Guajarati - Koholu, Punjabi - Golkaddu, Tamil - Pushni, Telgu - Durdagumuda, Arabic - Majdabh, Persian - Kadurumi, English -Ash Gourd, English Synonyms- Wax Gourd, Winter Melon, White Gourd, Tallow Gourd, White Pumpkin, Ash Pumpkin, Chinese preserving Melon, Pith Gourd and Hairy Melon (when immature).

Pharmacopoeial standard parameters

Pharmacopoeial research studies such as organoleptic characters, microscopical, macroscopical and physicochemical, TLC/HPLC., quality control and quality assurance parameters were carried out

1. **Organoleptic Evaluation:** Organoleptic evaluation refers to evaluation of formulation by colour, odour, taste, texture etc., using the sensory organs of our body. The organoleptic characters of the drugs samples were carried out based on the method described by Siddique *et al*. (1995).
2. **Powder Microscopy:** Take 3-5g powder drug sample was weighed, mixed with 50ml of distill water in a beaker and warmed gently in order to make complete dispersion in water. Then mixture was centrifuged and decanted supernatant. The sediment were washed several times with distilled water, centrifuged again and decanted the supernatant. Small quantity of the sediment was taken and mounted in 4645 eculariz, out of which another small quantity was taken in watch glass and a few drops of phloroglucinol and concentrated hydrochloric acid were added, mounted in 4645eculariz to locate lignified cells. The following characters in different mounts were observed (Wallis, 1987; Johansen, 1940).
3. **Physico-chemical analysis:** If the water content is high the drug can easily be deteriorated due to fungus, The ash content indicates the total amount of inorganic material after complete incineration and the acid insoluble ash is an indicative of silicate impurities might be due to improper washing of the drug. The alcohol and water soluble extractive indicates the amount of bioactive chemical constituents in a given amount of particular drug when extracted with respective solvent. Some of the useful tools in standardization of ASU herbal products such as moisture content of the powdered sample at 105ºC, ash values, acid insoluble ash, solubility in water and alcohol, pH values and bulk density and estimation of sugar etc., are useful tools were studies as per standard methods (Anonymous, 1987; 1998). Clearly shown in collected sample of North India Region U.P., U.K. and Bihar State Table.- 1.2. and 3 respectively.
4. **TLC/HPTLC finger printing analysis:** The drug samples (2gm) were soaked in chloroform and alcohol separately for 18 hours and refluxed for ten minutes on water bath and filtered through What man N0.1 filter paper. The filtrates were concentrated and made up to 10 ml in

volumetric flask with respective solvents (Saxena and Yadav, 1983). TLC/HPTLC finger print studies of chloroform and alcohol extracts of the drug were carried out using 4646eculariz plate precoated with silica gel 60 F254 (E. Merck) with CAMAG Linomat IV sample applicator. The chromatograms of both the extracts were taken using the solvent systems toluene: ethyl acetate (8 : 2 or 9 : 1) and toluene: ethyl acetate (8 : 2 or 6 : 4) for chloroform and alcohol extracts respectively. The plates were dried at room temperature and observed the spots at various wavelengths. The plates were scanned at 254 nm and to record the finger print spectrum after that same plates were visualized at UV-366 nm and derivatized with spraying of vanillin-sulphuric acid reagent and heated at 105° C till appeared coloured spots. (Khan *et al*.,2022 ; Sagar *et al*.,2020 and Wagner and Blad, 1996; Sethi, 1996).

5. **Quality assurance and quality control parameters:**
   Estimation of microbial load: The microbial load viz. total bacterial count (TBC), total fungal count (TFC), *Entero bacteriaceae*, *Escherichia coli*, *Salmonella* spp and *Staphylococcus aurous* were estimated as per standard method (WHO, 1998).

   Estimation of Heavy metals: The method used for the analysis of heavy metals like lead, cadmium, mercury and arsenic as per Guidelines of WHO. Heavy metals were analyzed by Atomic Absorption Spectroscopy (Anonymous, 1998) and AOAC (Anonymous, 2005). Details of the Instrument and operating parameters Thermo Fisher M Series, 650902 V1.27 model Atomic Absorption Spectrometer (AAS) was used for the analysis.

Analysis of Aflatoxins: Aflatoxins B1, B2, G1 and G2 were analyzed as per Official Analytical Methods of the American Spice Trade Association (ASTA), 1997. Aflatoxins were estimated by Kobra cell techniques using Agilent HPLC and CAMAG or Anchrom HPTLC instruments as per the method ASTA (Anonymous, 1997; Sagar *et al*.,2020).

Details of instrument and operating parameters High Performance Liquid Chromatography (Thermo Fisher) and CAMAG or Anchrom HPTLC were used for the analysis of aflatoxins. Column – Ultra C18, 250 X 4.6 mm, 5 µm particles; Mobile phase: Water: Acetonitrile: Methanol (65: 22.5: 22.5); Flow rate: 1 ml/min; Temperature: 35º C; Detector: Fluorescence detector at 360 nm; Injection run: 20 µl (Aflatoxins B1, B2, G1 and G2 mixture and test samples).

Analysis of pesticide residue: The method used for the analysis of pesticide residues was as per AOAC (Anonymous, 2005). Pesticide residues were analyzed by Gas Chromatography Mass Spectra (GC-MS) (Instrument- Thermo Scientific, Model –TSQ9000 or Agilent), detector-mass selective detector or Triple Quadrupole mass analyzer detector, column specification-DB-5MS or TG-5MS, carrier gas – helium, flow rate – 1ml/min, column length – 30 m, internal diameter – 0.25 mm, column thickness - 0.25 ìm).

The usage of ASU. herbal products along with higher safety margins, WHO has taken necessary steps to ensure quality assurance and quality control parameters with the modern techniques and application of suitable standards, (Anonymous,1998;Sagar *et al*., 2020; Meena et al., 2016).

RESULTS AND DISCUSSION

Organoleptic character of the formulated drug indicates that the drug is Morphologic features:

Macroscopic: The fruit is round and circular shape with greenish colour having whitish powdery mass on the uppers surface of the epidermis. The weight of the fruit may go up to 2-20 kg.

Microscopic identification: Outermost layer is epidermis which is elongated and parenchymatous in nature. The parenchyma cells are thin walled without intercellular space and are variable in their size. Vascular tissue shows xyllary elements and annular thickening of the vessels. Clearly shown in Fig.-3, 4, 5 and Fig.-6, 7, 8. Respectively

Maceration

Macerate of parenchymatous cell of epidermis without intercellular space, Annular thickening of xylem vessels, xyllary elements. clearly shown in Fig.- 4,6 and Fig.-7,8. Respectively

Fig.3

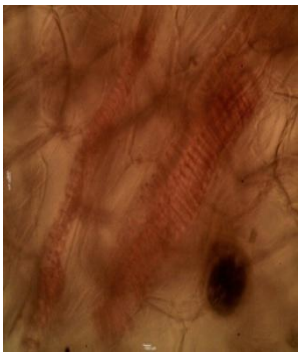


Fig.4

Fig.5

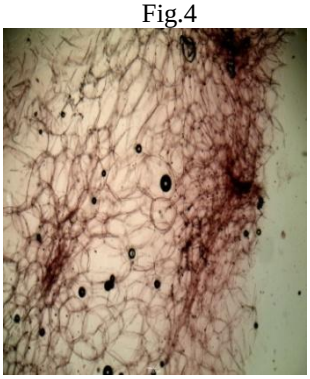


Fig.6)

Fig.7

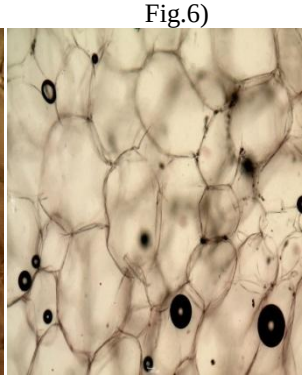


Fig.8

Fig.3 Petha pulp (surface view showing parenchyma cells)
Fig.4 Petha pulp (Xyllary elements)
Fig.5 Petha pulp (Epidermal cells and parenchyma)
Fig .6 Petha pulp (Thin layer parenchyma)
Fig.7 Petha pulp (Annular thickening of vessels)

Fig.8 Petha pulp (Parenchyma cells without intercellular space)

Table1 Active Physico-chemical Compositions

S.NO.	Petha (Ash Gourd) Fruit part	Phsico-chemical Composition of Petha	Resulted values	References
01	Total Fruit Contained	Moisture (%)	96.20	Gupta *et al*., 2019; Bimakar *et al*., 2012
02		Protein (g)	12	
03		Fat (g)	ND	
04		Carbohydrate (g)	3.96	
05		Zinc (mg)	0.6	
06		Iron (mg)	11.8	
07		Calcium (mg)	30	
08		Fiber (g)	2.9	

Where: ND= Not detect

Table 2 Active Phyto-Chemical Constituents Compositions

S.NO.	Active Phyto-Chemical Constituents	Resulted Values	References
01	Total phenol	28.36,%	Gupta *et al*., 2019; Devaki and Premavalli, 2012; Nadhiya and Vijayalakshmi, 2014; Siddhuraja and Beckers, 2001
02	Antioxidant	12.60,%	
03	Acidity	0.210,%	
04	Total volatiles	0.002,%	
05	Alcohol	4.60,%	
06	Total Flavonoid	1.67,%	
07	Tannin	10.4 ± 0.02	

Table 3 Chemical identification tests: Physico-chemical parameters

S. No.	Parameters	Resulted Values		
		P-1	P-2	P-3
1.	Foreign Matter (%)	NIL	NIL	NIL
2.	Loss in weight on drying at 105^0 C (%)	3.80	3.86	3.90
3.	Total Ash (%)	6.10	6.23	6.30
4.	Acid insoluble ash (%)	0.30	0.32	0.35
5.	Ethanol Soluble Extractive (%)	2.44	2.50	2.57
6.	Water Soluble Extractive (%)	5.50	5.58	5.62
7.	pH of 1% aqueous solution	5.90	5.92	5.95
8.	pH of 10% aqueous solution	5.32	5.36	5.38

Table 4 R_f values of alcohol extract

Solvent system	R_f Values		
	UV Light - 254nm	UV Light - 366nm	Visible light V-S Reagent
Toluene - Ethyl acetate (9: 1)	ND	ND	0.20, (Gray)
	ND	ND	0.27, (Pinkish purple)
	ND	ND	0.30, (Pinkish purple)
	ND	ND	0.39. (Pinkish Gray)
	ND	ND	0.49, (Pinkish purple)
	ND	ND	0.59, (Pinkish purple)

Where: ND= Not detect

Table 5 R_f values of alcohol extract

Solvent system	R_f Values		
	UV Light - 254nm	UV Light - 366nm	Visible light V-S Reagent
Toluene - Ethyl acetate (9: 1)	ND	ND	0.20, (Gray)
	ND	ND	0.28, (Pinkish purple)
	ND	ND	0.31, (Pinkish purple)
	ND	ND	0.41. (Pinkish Gray)
	ND	ND	0.47, (Pinkish purple)
	ND	ND	0.59, (Pinkish purple)

Where: ND= Not detect

C / HPTLC Finger printing analysis

TLC/ HPTLC finger printing profiling of Chloroform and Alcohol extract of 2g of sample with 20ml of alcohol separately and reflux on water bath for 30min. Filter and Concentrate the filtrate up to 10 ml (approx.) on water bath and apply the alcohol extract was spotted on silica gel "G" plate / precoated aluminum TLC plate of silica gel 60 F_{254}using HPTLC automatic sample applicator. Develop the plate in Toluene - Ethyl acetate (9: 1) solvent system. Allow the plate to dry in air and examine under UV (366nm).No major spot is observed. Under UV (254nm), no major spot is observed. Dip the plate in 1% Vanillin - Sulphuric acid reagent followed by heating at 105^0C for 5 minutes and examine under visible light. Observe 06 major spots at R_f 0.20(grey), 0.28, 0.31(pinkish purple), 0.41(Pinkish grey), 0.47 & 0.59(pinkish purple). Chloroform Petha, *Beninca hispida* (Thunb.) extract clearly shown in Fig.2. and Table - 4, respectively.

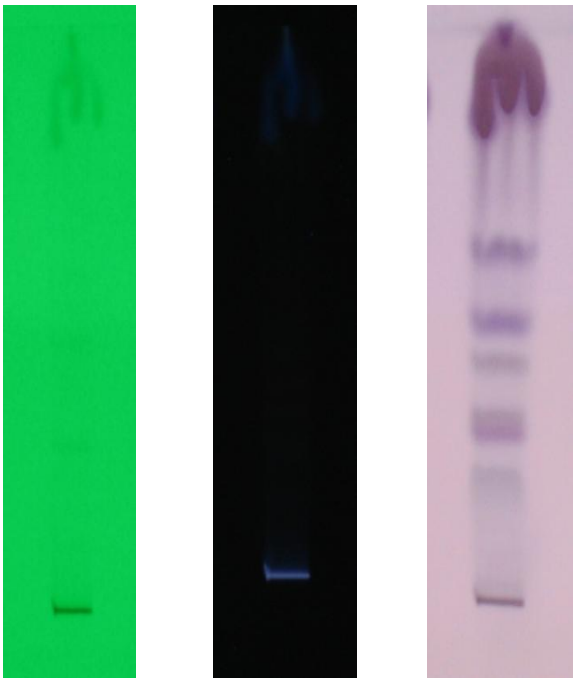
Apply *Ethanol* extract on percolated aluminum TLC plate of silica gel 60 F_{254} using HPTLC automatic sample applicator. Develop the plate in Toluene - Ethyl acetate (9:1) solvent system. Allow the plate to dry in air and examine under UV (366nm).No major spot is observed. Under UV (254nm), no major spot is observed. Dip the plate in 1% Vanillin – Sulphuric acid reagent followed by heating at 105^0C for 5 minutes and examine under visible light. Observe 06 major spots at R_f 0.20(grey), 0.27, 0.30(pinkish purple), 0.39Pinkish grey), 0.47 & 0.59(pinkish purple). HPTLC. fingerprinting separation of separated spots of fresh Fruit of Alcohol Petha, *Beninca hispida* (Thunb.) extract clearly shown in Fig.3. and Table - 5, respectively.

Quality Assurance and Quality Control Parameters

Detection and validation of Pharmacopeial quality parameters of test samples in order to assess the quality of drug samples. The analysis of microbial load present in the drug showed that the total bacterial count (TBC) and total fungal count (TFC) was revealed 600 and 500 cfu/gm. The detection of the microbial load was under the permissible limits of WHO guideline. the estimation of microbial load viz. total bacterial count (TBC), total fungal count (TFC), *Entero bacteriaceae*, *Escherichia coli*, *Salmonella* spp and *Staphylococcus aurous* were analyzed and found to be in permissible limit. The results are shown in (Table - 4). The heavy metal such as lead was present within the permissible limit where as cadmium; mercury and arsenic were not detected from the drug samples. The results are shown in (Table-5). The studies of other parameters like estimation of afltoxins such as B1, B2, G1 and G2 The results are shown in (Table- 6) and pesticide residue such as organo chlorine group, organo phosphorus group, alachlor, aldrin, chlordane, DDT, endosulfan, heptachlor, lindane and malathion etc. were not detected from the drug, The results are shown in (Table -7) respectively.

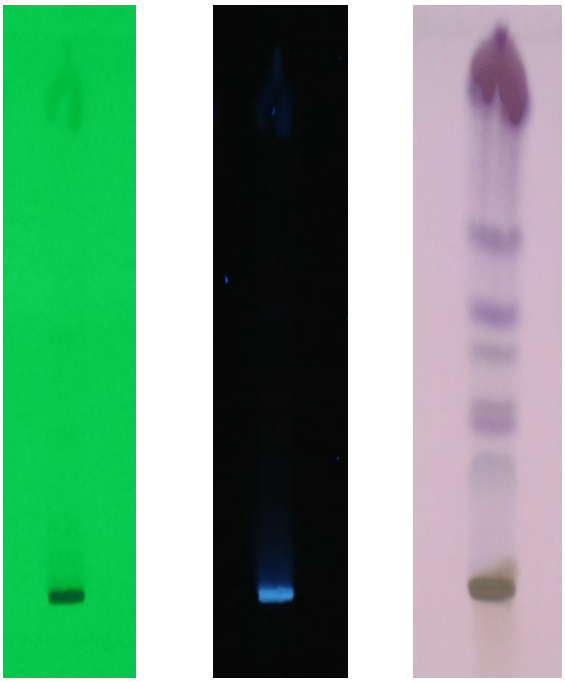
Active Phyto-chemical constituents

Active phytochemical constituents have been reported & investigated in fruit of Petha, *Beninca hispida* (Thunb.). The seeds contain tannins, carotenoids, oxalates, and phytate. (Nagarajaiah *et al*,.2015). proteins.(Churiyah *et al*., 2009) carbohydrates, phenolic compounds, amino acids, flavonoids, sterols. (Qadrie *et al*.,2009), glycosides, alkaloids, fixed oils and fats, phenolic compounds, steroids.(Hemant *et al*.,2014), and unsaturated fatty acids. (Bimakr *et al*., 2016), The peel contains alkaloids, saponins, steroids, carbohydrates, flavonoids.(Rana *et al*., 2012), *B. hispida* is rich in phenolic compounds. Several other bioactive compounds present in it are isomultiflorenyl acetate, isovitexin, 1-sinapoylglucose, multiflorenol, 5-gluten-3-*β*-ylacetate, alnusenol, and benzylalco lcohol-*O*-*α*-l-arabinopyranosyl- (1-6)-*β*-d-glucopyra noside. (Du *et al*., 2005; Islam *et al*.,2021), Flavonoids, glycosides, sacchrides, proteins, carotenes, vitamins, minerals, uronic acid, multiflorenol, isomultiflorenyl acetate, stigmasterol, α-spinasterol, β-sitosterol, daucosterol, arbutin, nicotinic acid, (+)- pinonesinol and ethyl β-D-glucopyranoside. (Islam *et al*., 2021 Gupta *et al*., 2019)



UV 254nm UV 366nm Visible Light (After derivatization)

Fig. 2 HPTLC pic. of Chloroform extract of P



UV 254nm UV 366nm Visible Light (After derivatization)

Fig.3. HPTLC pic. of Ethanol extract of Petha

The investigated drug *Beninca hispida* (Thunb.) - Petha was

Table-6: Analysis of Microbial load

S.N0.	Parameter Analyzed	Results	WHO Limit
1	Total Bacterial Count	600 cfu/gm	10^5cfu/gm
2	Total Fungal Count	500 cfu/gm	10^3cfu/gm
3	*Escherichia coli*	Absent	Absent
4	*Salmonella typhai Spp.*	Absent	Absent
5	*Staphylococcus aurous*	Absent	Absent

Table-7: Estimation of Heavy Metals

S.N0.	Parameter Analyzed	Results	WHO Limit
1	Lead	2.52ppm	10ppm
2	Cadmium	0.03ppb	0.3ppm
3	Mercury	Not detected	1.0ppm
4	Arsenic	0.09 ppm	3.0ppm

Table-8: Estimation of Aflatoxins

S.N0.	Parameter Analyzed	Results	WHO Limit
1	Aflatoxins, B1	Not detected	0.5ppm
2	Aflatoxins, B2	Not detected	0.1ppm
3	Aflatoxine, G1	Not detected	0.5ppm
4	Aflatoxine, G2	Not detected	0.1ppm

Table-9: Estimation of Pesticide Residues

S.N0.	Parameter Analyzed	Results	WHO Limit (mg/kg)
1	DDT (all isomers, sum of ρ, ρ'-DDT, α, ρ' DDT, ρ, ρ'-DDE and ρ, ρ'-TDE (DDD expressed as DDT)	Not detected	1.0
2	HCH (sum of all isomers)	Not detected	0.3
3	Endosulphan (all isomers)	Not detected	3.0
4	Azinphos-methyl	Not detected	1.0
5	Alachlor	Not detected	0.02
6	Aldrin (Aldrin and dieldrin combined expressed as dieldrin)	Not detected	0.05
7	Chlordane (cis& tans)	Not detected	0.05
8	Chlorfenvinphos	Not detected	0.5
9	Heptachlor (sum of heptachlor and heptachlor epoxide expressed as heptachlor)	Not detected	0.05
10	Endrin	Not detected	0.05
11	Ethion	Not detected	2.0
12	Chlorpyrifos	Not detected	0.2
13	Chlorpyrifos-methyl	Not detected	0.1
14	Parathion methyl	Not detected	0.2
15	Malathion	Not detected	1.0
16	Parathion	Not detected	0.5
17	Diazinon	Not detected	0.5
18	Dichlorvos	Not detected	1.0
19	Methidathion	Not detected	0.2
20	Phosalone	Not detected	0.1
21	Fenvalerate	Not detected	1.5
22	Cypermethrin (including other mixtures of constituent isomers sum of isomers)	Not detected	1.0
23	Fenitrothion	Not detected	0.5
24	Deltamethrin	Not detected	0.5
25	Permethrin (sum of isomers)	Not detected	1.0
26	Pirimiphos methyl	Not detected	4,0

CONCLUSION

assurance of its genuine quality and free from any impurities or hazardous, toxic contamination according to the drug quality

research, botanical identification, Pharmacognosy, physic chemical and quality control results studies data's basis. The ranges of all the botanical identification, Pharmacognosy, physicochemical constants used for the quality analysis of the entire B H. plant part are normal. Numerous secondary metabolites have been detected. As the evaluated of resulted parameters which certainly provides validation that the drug is safe for internal use. HPTLC fingerprinting also contributes for maintaining its authenticity. As well as its potent quality, safety and toxicity evolution of studies. B H. may be therapeutically used in many health disorders and supported data's of B H. can be helpful to incorporated of pharmacopoeial standard monograph. However; further studies on the isolation and characterisation of these substances may still be conducted and expected to advance comprehend confirmation of the *In-vitro* or *In-vivo* detailed mode of action upon animal trial model of BH.- Petha.

Acknowledgment

The authors are extremely thankful to the Dr. N. Z. Ahmed, Director General, CCRUM, New Delhi, under ministry of AYUSH, Govt. of India for his valuable guidance, encouragement and necessary research facilities to carry out the research studies as well as also thankful to our all of dedicated research staff team of the research Institute for providing full cooperation and valuable support to complete this research work.

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How to cite this article:

Pawan Kumar Sagar, N. Z. Ahmed, R.P.Meena and S. Kashyap. (2024). Pharmacopeial standard development, pharmacognosy, hptlc. fingerprinting and physicochemical research studies of beninca hispida (thunb.). *Int J Recent Sci Res.*15(04), pp.4644-4651.

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