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ORIGINAL ARTICLE **OPEN ACCESS**

Pharmacognostical Standardization and Isolation of Biomarker from *Cucumis dipsaceus, Momordica diocia* and *Momordica charantia Var. Muricata*

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ABSTRACT
The present investigation evaluates Pharmacognostical standardization and Isolation of biomarkers from Cucurbitaceae family plants Cucumis dipsaceus, Momordica diocia and Momordica charantia var. muricata. Here, Pharmacognostic and Physicochemical studies were perform like morphology, microscopy, ash value, loss on drying, swelling index and extractive value as per WHO guidelines. Extraction performed by using soxhlet extractor successively in solvent petroleum ether, chloroform, methanol, ethyl acetate and water. Different extract were prepare and tested for phytochemical screening. Biomarkers from extract were isolated by column chromatography then identified and confirmed using TLC, melting point determination, UV and IR–Spectroscopy method. This study revealed the presence of antidiabetic phytochemical in plant extracts like Charantin, Cucurbitacin. The present study confirms the further explored to the comparative biological activity of isolated compound for further confirmation.
Keywords*: Extraction, Isolation, chromatography, spectroscopy.*

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INTRODUCTION

Herbal medicine becomes more popular because less side effect, minimum toxicity, effective in chronic disorders. Indian people mostly depend on Indian system of medicine called Ayurveda –an ancient science of life [1]. Acceptance of herbal drug in modern system of medicine their quality assessment is important, for that different methods of standardization were consider justifying the quality of herbal drug. In proper identification of drug organoleptic, pharmacognostic, physicochemical and phytochemical evaluation is necessary [2]. Many compounds isolated from plant sources have been reported to show different activity. By focusing on same, the herbal material was standardized for pharmacognostical, physicochemical and phytochemical parameters.

Cucumis dipsaceus (Family-*Cucurbitaceae*): Local Name- Wild Cucumber and Vernacular Names: Hedgehog Cucumber, Hedgehog Gourd. It is fibrous rooted plant producing stem up to 2 meters long that scramble over the ground or climb. Chemically contain alkaloids, flavonoids, tannins, resins, steroids. Seeds- cooked. Pale brown seeds 3-5 mm long use as a poultice to treat wound. Juice from the fruit is use as an antidote for poisoning, but has to be supplemented by drinking fresh milk. Fruits used for diarrhoea, stomach pain, constipation, meningitis. Pharmacologically fruit extract shows antibacterial, cytotoxic and antitumour activity [3, 4].

Momordica diocia (Family-*Cucurbitaceae*): Synonym: kartoli, kantola, teasle gourd, small bitter gourd is a vegetable climber. The green fruit is extensively used as vegetable for cooking. Chemically contain alkaloid, flavonoids, glycosides, tannins, saponins and amino acids, triterpene. Use as immunostimulant and antiseptic. Its fruit are used as vegetable, Use in India as a folk remedy for diabetes also use as antimalerial, antiallergic, antioxidant and hepatoprotective activity [5].

Momordica charantia var. muricata: (Family-*Cucurbitaceae*): Synonym: *Momordica muricata* Wild is Monoecious Climber and having glabrous unbranch tendrils. Pubescent stem leaves are cordate, hairy and 1.5-3.0 cm long, yellow colour pedicellate flower 2.5-3 cm in length.

Fruits are 2-7 cm long 1-3 cm broadly ovate, seeds are elliptic ovate 1-1.5 cm long and 6-7 cm broad. Chemically contain antidiabetic compound triterpenoid, charantin, alkaloids, momordicine, glycosides [6, 7].

For The present study, three plants are selected from *Cucurbitceae* family and investigation evaluates Pharmacognostical standardization and Isolation of biomarkers .

MATERIAL AND METHODS

Collection and Authentication of Plant: Selected part of plants collected from local area of Pune District namely Rajuri from Maharashtra. The material was Identified and Authenticated from Botanist Dr. S. S. Ranhandgule , Professor (H.O.D BOTANY) Balasaheb Jadhav College of Art, Commerce & Science , Ale Junnar, Pune Maharashtra Dated 20/11/2018. Herbarium specimen has been preserved in laboratory voucher specimen no. 857 for *Momordica diocia*, specimen no.858 For *Cucumis dipsaceus* and specimen no.859 For *Momordica charantia* var. *muricata*.

Pharmacognostic Evaluation:

Morphological and Microscopical Evaluation: The morphological characteristics of selected parts of plants such as colour, odour, size, shape, taste were carried out. For microscopy Free hand sections of the Fruits of *Momordica diocia*, fruit, seeds of *Momordica charantia* var. *muricata* and seed, fruits of *Cucumis dipsaceus* was taken and stained with different staining reagent to confirm its lignifications. And it was observed under magnification [8].

Powder Microscopy: Needle tip moisten with water, deep into crude powder. Place the needle tip into drop of water present on glass slide, mixed thoroughly and cover with cover slip after lightly pressed it. Remove the excess water by using filter paper from the margin of cover slip and it was observed under magnification[9].

Physicochemical Evaluation:

Foreign Organic Matter Determination: 100gm powder sample was taken spread in
thin layer and detect the foreign matter with using lens (6x) or unaided eye. Separate the foreign matter, weigh it and calculate the percent present[2].

Moisture Content Determination:

Loss on drying: 10 gm powdered drug place in evaporating dish and dry at temp.105˚C for 5 hr. At one hour of interval take weighing and calculate the constant difference of two successive weighing after drying difference show not more than 0.01 gm [2].

Extractive Value Determination:

A) **Alcohol Soluble Extractive Value**: 4gm coarsely powder air dried drug macerate with 100 ml (90%) ethanol for 24 hrs. Shake frequently for first 6 hr. and allowed to stand for 18 hrs. 25 ml of filtrate was evaporating on water bath. Dry in hot air oven cool in desiccator and weigh.
The percentage of alcohol soluble extractive value was calculated using formula.
% of extractive value= B-A×4×100/W
A: empty dish weight (gm)
B: weight of dish +residue (gm)
W: weight of drug taken (gm)

B) **Water Soluble Extractive Value**: 4 gm coarsely powder drug macerate with 100ml of 5% Chloroform -water for 24 hrs. Shake frequently for first 6 hr. and allowed to stand for 18 hrs. 25 ml of filtrate was evaporating on water bath. Dry in hot air oven cool in desiccators and weigh. The percentage of water soluble extractive value was calculated using formula [8].
% of extractive value= B-A×4×100/W

Ash Value Determination:

A) **Total Ash Value Determination:**
3gm air dried powder drug taken in silica crucible and incinerate at temp. Not exceeding 450˚c until all carbon is burn off, cool. Calculate the percentage of total ash value with reference to the air dried powder drug.

B) **Acid Insoluble Ash Value**: The obtained total ash was boil for 5 min in 25 ml dil. HCL. Filter the residue was collect on ash less filter paper, wash with hot water, ignite and weigh. The percentage of acid insoluble ash was calculated with reference to air dried drug.

C) Water Soluble Ash Value: The obtained total ash was boil for 5 min in 25 ml dil. HCL. Filter the residue was collect on ash less filter paper, wash with hot water, and ignite. Insoluble matter was subtracted from the weight of the ash. The difference in weight represent the water soluble ash .The percentage of water soluble ash was calculated with reference to air dried drug [9].

Swelling Index Determination: 1gm seeds transfer in 150 ml measuring cylinder, add 50 ml distilled water kept aside for 24 hr. with occasional shaking , the volume occupied by seeds after 24 hour was measured [2].

Extraction of Phytoconstituents:

100 gm of coarsely powder air-dried material successively extracted with different solvent 350 ml of petroleum ether (40-60°C) for 6 hr., 350 ml of chloroform (40-50°C) for 5-6 hr., 350 ml of methanol (60°C) for 12-13 hr., 350 ml ethyl acetate at temperature (60-70°C) for 6 hr. and 350 ml of water at temperature (80°C) for 6 hr. in soxhlet extractor. Separate the extract and solvent was recovered in rotary evaporator. Cool and store the extract in desiccator and calculate the percentage yield of extract [8].

Preliminary Phytochemical Screening on Extract [10]

Different chemical test was done on the extracts to determine the presence of alkaloids, carbohydrate, glycoside, saponin, protein, amino acid, phytosterol, fixed oil, fats, phenolic compound and tannins.

Thin Layer Chromatography Study on Extract:

Thin layer chromatography was performed on ethanolic extract of *Cucumis dipsaceus*, methanolic, chloroform extract of *Momordica diocia* and ethanolic extract of *Momordica charantia* var. *muricata* using pre coated TLC plate (silica gel GF-254). After application of sample solution the plates were kept in mobile phase chamber for TLC development as per given in table 1. Then the separated spot was observed and calculate the R_f value using formula.

R_f value: sample front distance from origin /solvent front distance from origin

Table 1: TLC Profile of Extracts

Sr. no.	Plant Extract	Test Solution	Stationary Phase	Mobile Phase	Visualization
1.	*Cucumis dipsaceus [11]*	Ethanolic Extract	Silica gel G Plate	Chloroform: Methanol (95:10)	Day light
2.	*Momordica diocia* [12]	Methanolic Extract & Chloroform Extract	Silica gel G Plate	Chloroform:Methanol (95:10)	Day light
3.	*Momordica charantia var. muricata* [6]	Ethanolic Extract	Silica gel G Plate	Methanol: Benzene (2:8)	UV Chamber (254 nm)

Isolation of active chemical compound:

Cucurbitacin isolation:

By general solvent extraction:

50 gm Powdered drug macerated with equal volume of ethanol (100ml) and lead acetate (100ml) for 7 days. Filtrate treated with potassium dihydrogen phosphate to give lead precipitate then partition with aqueous phase and chloroform, the chloroform extract give Cucurbitacin after purification by chromatographic technique [13].

By column chromatography method:

Column chromatography –isocratic elution technique fallowed using methanolic extract (10gm) with mobile phase: Chloroform and ethyl acetate (9:1). At the base of column, place glass wool. Slurry was made using activated silica and hexane which is then transfer in column, in small portion with tapping to remove air bubbles. Small quantity of eluent should be in top of column for prevention of drying and cracking in column. Then add filter paper disc, add methanolic extract. The column was eluted with solvent methanol: water (2:8). Fraction were collected and checked by TLC technique [14].

Charantin isolation:

By general solvent extraction:

Fruit powder (100 gm) Extracted with Pet. Ether (600 ml), Marc taken further extracted with 80 % ethanol (500 ml) for 6 hr. Filter, filtrate basified with KOH solution, up to pH 10 (keep for 48 hr.) Resulting solution extracted diethyl ether. Then Wash with water then hydrochloric acid and again with water. Then ether portion mix with anhydrous sodium sulphate. Filter conc. get crude Charantin residue [15].

By column chromatography method:

10 gm Methanolic extract suspended in water and then sequentially fractionated with hexane and ethyl acetate thrice. Then fallowed column chromatography eluted with chloroform: methanol (50:50) gives yield of Charantin fraction [16].

Identification of isolated compound:

Confirmatory test on isolated extract:

Charantin: Liebermann-Burchard test : sample mixed with 1ml chloroform and 1ml acetic anhydride and add 1 drop of conc. Sulphuric acid, blue green to red orange colour confirm presence of Charantin[15].

TLC of isolated compounds:

Isolated compound shows single compound on TLC plate after development using sample solution and mobile phase as per given in table 2. It gives same R_f value with standard it indicates presence of marker compound Cucurbitacin and Charantin.

Table 2: TLC Profile of isolated compound

Compound	Test Solution	Stationary Phase	Mobile Phase	Visualization
Cucurbitacin[11]	10mg in 10 ml chloroform	Silica gel G plate	Chloroform :methanol (95:10)	Day light
Charantin[15]	10mg in 10 ml Ether	silica gel G plate	Benzene : Methanol (2:8)	UV chamber (254 nm)

Melting point determination: It was done by capillary method [14, 17].

Spectroscopic Evaluation of Isolated Compound:

Ultra Violet Spectroscopy:

Procedure:

Take spectra on UV-visible spectrophotometer shimadzu using wavelength 200-400 nm. 100 µg/ml solution of Charantin in methanol was prepared as stock solution. And methanol is used as blank. From the above stock solution 0.5ml dissolved in solvent to make up volume up to 25 ml, to get 2ppm solution. Same 1ml from stock solution dissolved in solvent to make up volume up to 25 ml to get 4 ppm solution. 1.5 ml from stock solution dissolved in solvent to make up volume up to 25 ml, to get 6 ppm solution.

2 ml from stock solution dissolved in solvent to make up volume up to 25 ml to get 8 ppm solution and 2.5 ml from stock solution dissolved in solvent to make up volume up to 25 ml to get 10 ppm solution [15].

Infra Red spectroscopy:

By using KBR disc method 1 mg of isolated compound triturated with 70 mg potassium bromide in mortar. Triturate until fine powder was obtain. The powder was placed in hydraulic press to obtain pellet. Observe the IR Spectra for the compound in range of 400-4000cm^{-1} [14].

RESULT AND DISCUSSION

Pharmacognostical Evaluation:

Morphological Evaluation: The morphological studies of selected medicinal plants fruits and seeds of *Cucumis dipsaceus*, fruit and seeds of *Momordica diocia* and seeds of *Momordica charantia* var. *muricata* was determined and shown in table 3.

Table 3: Morphological Evaluation

S.No	Name of plant /drug	Plant part	Size	Shape	Colour	Odour	Taste
1.	*Cucumis dipsaceus*	Fruit	8.5 cm long and 5 cm width	Ellipsoid or spherical densely covered with spines	Green	Unpleasant	Bitter
		Seed	03-0.4cm long & 0.1-0.2 cm width	Egg-shaped	Yellow	Unpleasant	Bitter
2.	*Momordica diocia*	Fruit	2.3 cm long, and 1.5 cm width	ellipsoid, shortly beaked, densely with soft spines	Green – yellow	Unpleasant	Bitter
		Seed	1-1.2 cm length & 0.3-0.4cm width	Rounded cover with red pulp	Cream	Unpleasant	Bitter
3.	*Momordica charantia* var. *muricata*	Seed	1.0-1.1cm long & 0.3-0.4cm width	Base and apex subtridentate	Cream	Bitter	Bitter

Cucumis dipsaceus:

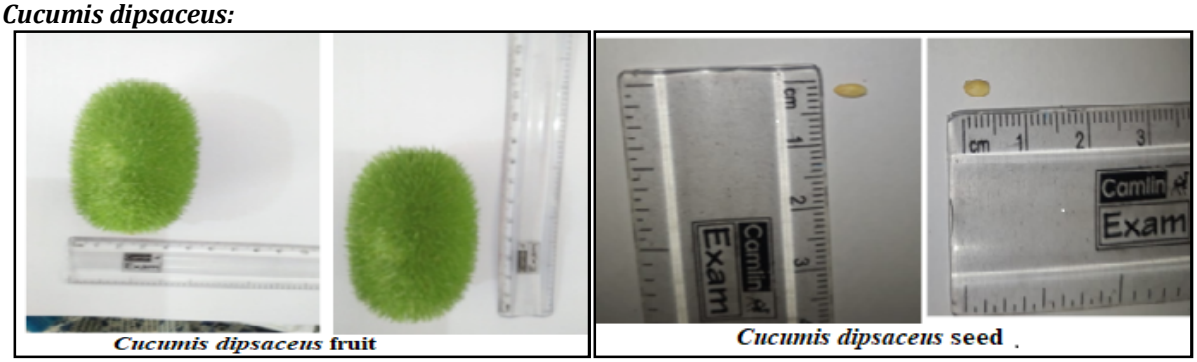

Fig. 1: Morphology *Cucumis dipsaceus*

Momordica diocia:

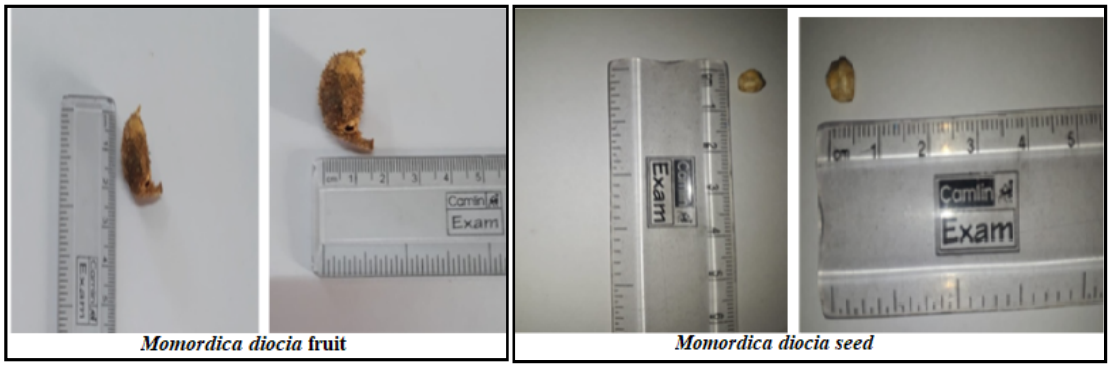

Fig. 2: Morphology *Momordica diocia*

***Momordica charantia* var. *muricata*:**

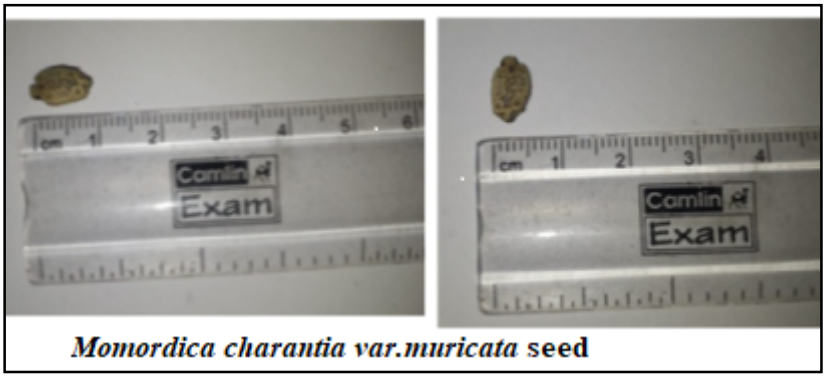

Fig. 3: Morphology *Momordica charantia* var. *muricata*

Microscopical evaluation:

Cucumis Dipsaceus:

Microscopically the seeds of *Cucumis Dipsaceus* contain endosperm, epidermis,testa, and cotyledons as shown in fig. 4. Microscopically fruit shows endocarp and trichomes endosperms and mesocarp under 4x and 10x magnifying lens as shown in fig. 5.

Seed:

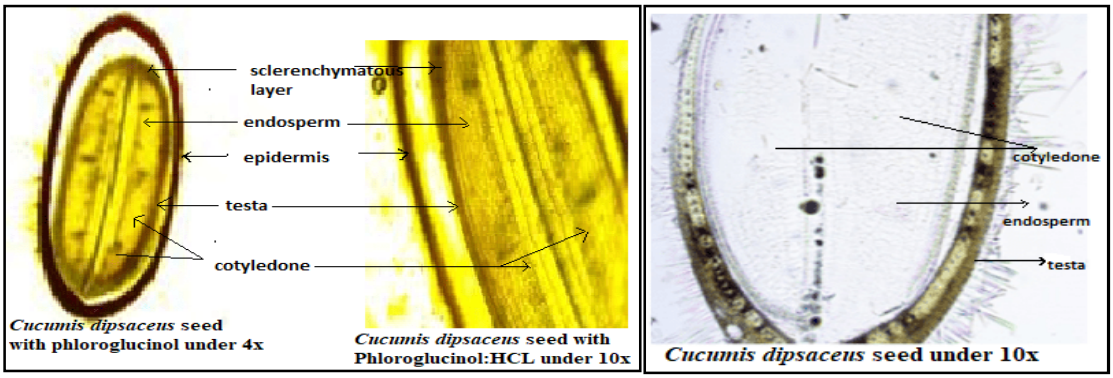

Fig. 4: Microscopy of *Cucumis dipsaceus* seed

Fruit:

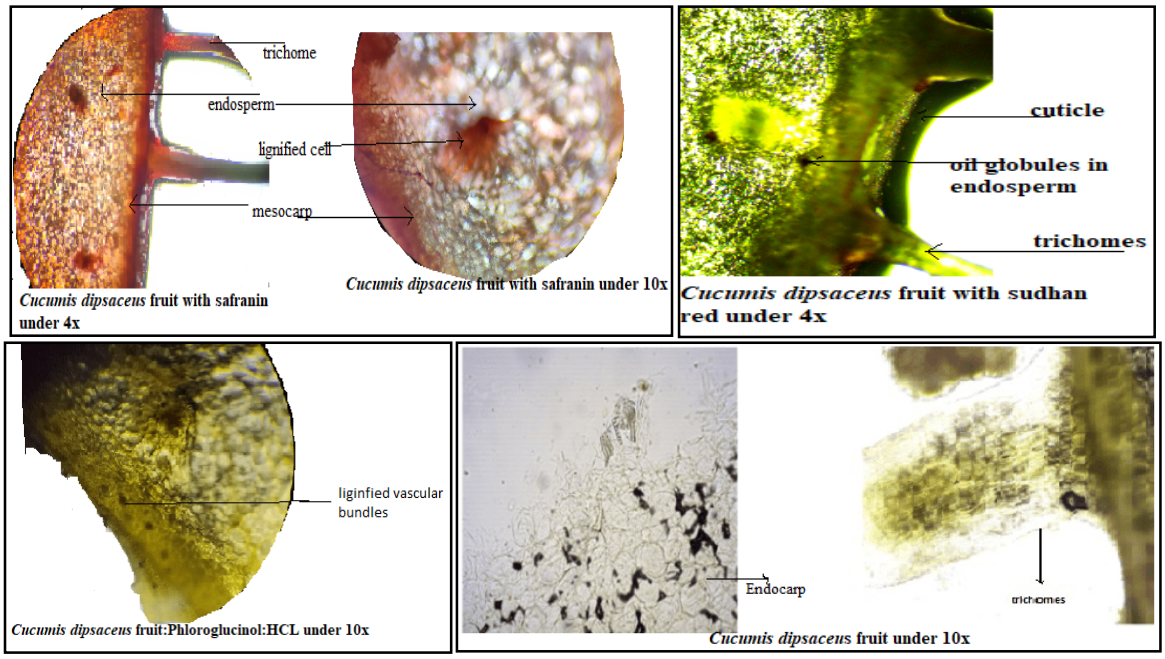

Fig. 5: Microscopy of *Cucumis dipsaceus* fruit

Momordica charantia muricata:

Microscopy of *Momordica Charantia Muricata* seeds shows abundant cotyledon, endosperm and sclerenchymatous layer as shown in fig. 6. Fruit shows endosperm and lignified vascular bundles also shows mesocarp under 4x and 10x magnifying lens as shown in fig. 7.

Seeds:

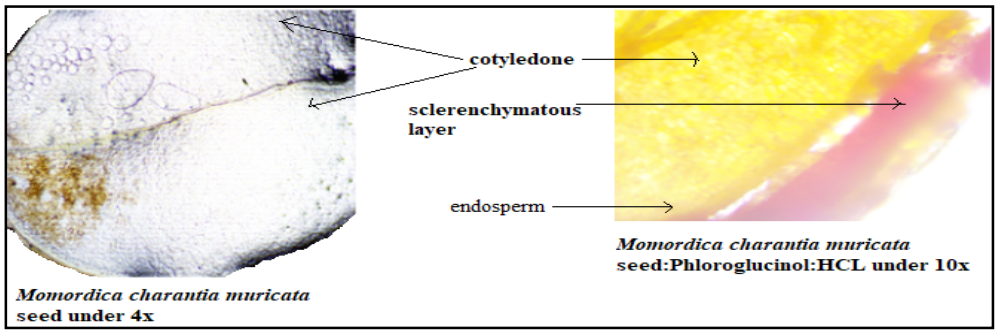

Fig. 6: Microscopy of *Momordica charantia muricata* seed

Fruit:

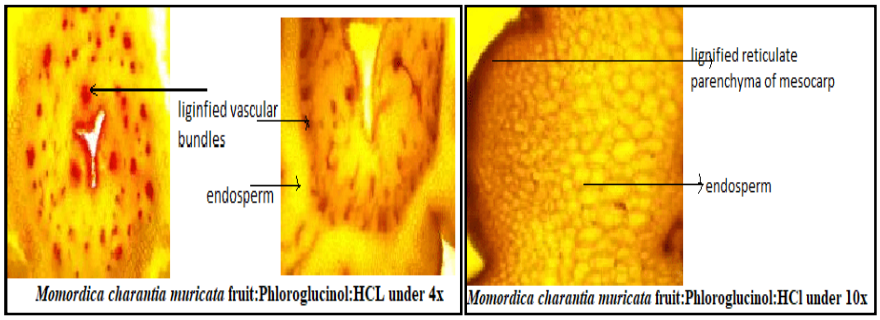

Fig. 7: Microscopy of *Momordica charantia muricata* fruit

Momordica Diocia: Fruit

Microscopically *Momordica diocia* fruit shows epicarp, mesocarp, trichome and endosperm under 4x and 10x magnifying lens as shown in fig. 8.

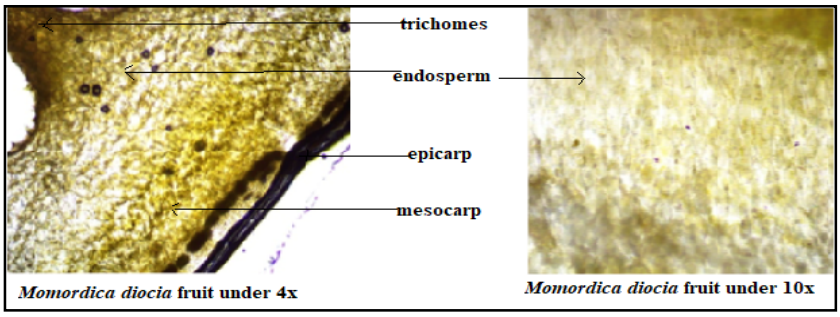

Fig. 8: Microscopy *Momordica diocia* Fruit

Powder Microscopy:

Momordica Charantia Muricata powder under 10x and 40x magnification shows vessel ,phloem fibers, endosperm, sclerenchyma, calcium oxalate crystal, reticulate parenchyma of mesocarp, epidermal cells of pericarp, mesocarp, fibers, multicellular covering trichomes, spiral vessel as shown in fig. 9.

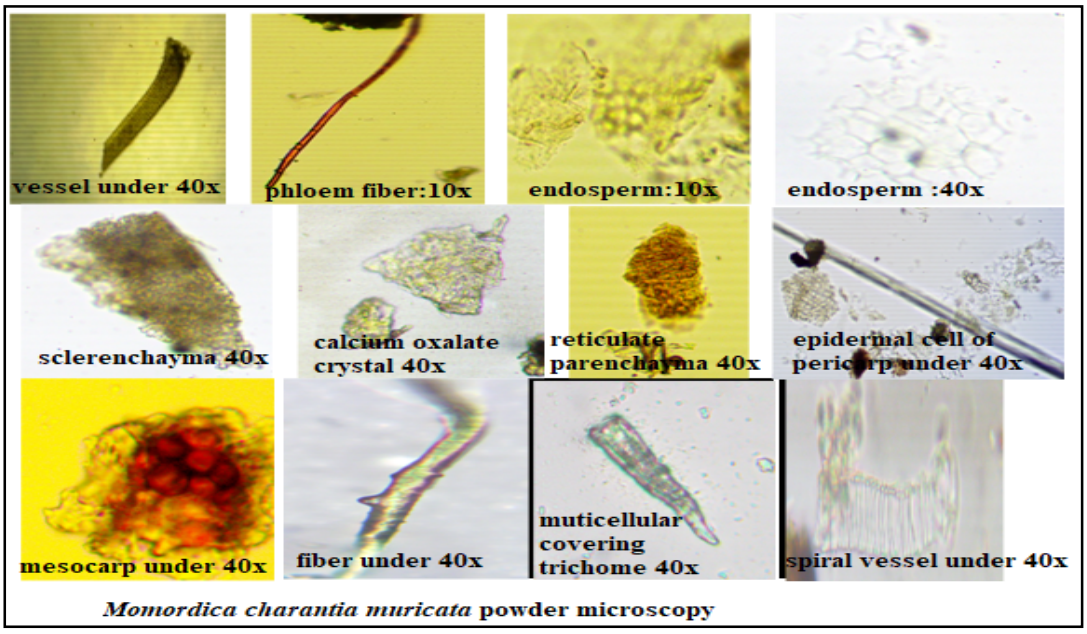

Fig. 9: Powder Microscopy *Momordica charantia*

Momordica diocia powder under 10x and 40x magnification shows Xylem vessel, non-glandular trichome, vessel, epidermis, strach grains, perisperm, Calcium oxalate crystals, lignified sclenchymatous layer containing group of fusiform fiber, running wavy , glandular trichomes as shown in fig. 10.

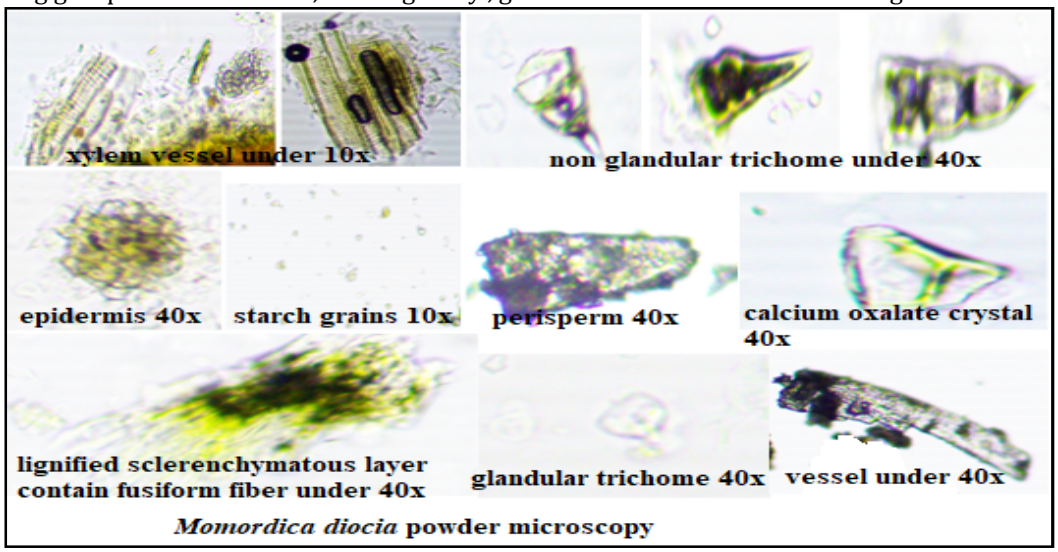

Fig. 10: Powder Microscopy *Momordica diocia* Fruit

Cucumis dipsaceus powder under 10x and 40x magnification shows trichomes, mesocarp, aleurone grains, fiber, starch grains and epidermis as shown in fig. 11.

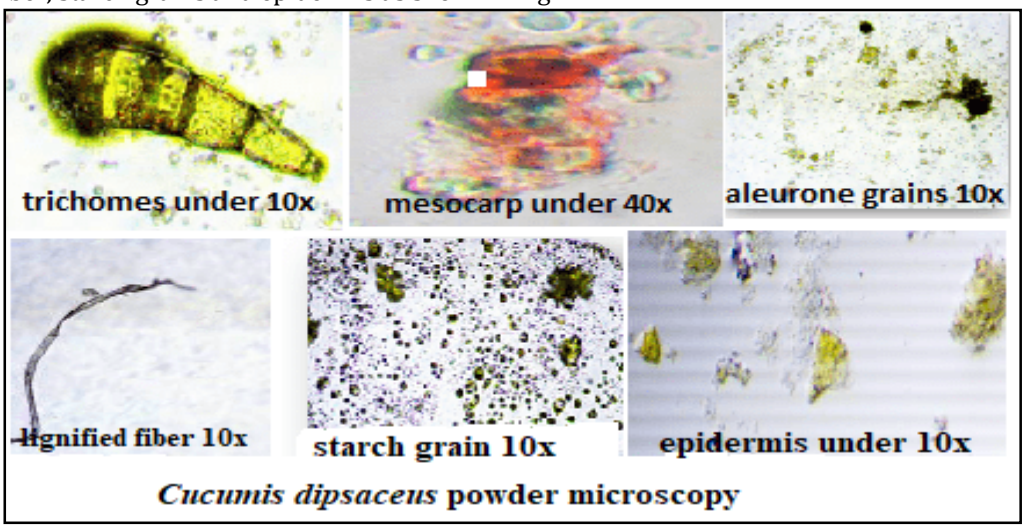

Fig. 11: Powder Microscopy *Cucumis dipsaceus*

Physicochemical Evaluation:

Foreign Organic Matter Determination:

Foreign Organic Matter for *Cucumis dipsaceus, Momordica diocia* and *Momordica charantia* var. *muricata* was determined and obtained result as shown in table 4.

Sr. No.	Name of Plant	Foreign Organic Matter (%)	
		Result	Standard value
1.	*Cucumis dipsaceus*	6.0	-
2.	*Momordica diocia*	4.0	NMT 5.0 [18]
3.	*Momordica charantia muricata*	0.01	NMT 1 [12]

Table 4: Foreign Organic Matter

Moisture Content Determination:

Moisture Content Determination for *Cucumis dipsaceus, Momordica diocia* and *Momordica charantia* var. *muricata* was determined obtained result as shown in table 5.

Sr. No	Name Of Plant Crude Drug	Moisture Content (%)	
		Result	Standard value
1.	*Cucumis dipsaceus*	8.16	NMT 8.4 [19]
2.	*Momordica diocia*	7.8	NMT 8.4 [18]
3.	*Momordica charantia var. muricata* (wild)	6.9	NMT 8.9 12]

Table 5: Moisture Content

Extractive Value Determination:

Extractive value Determination for *Cucumis dipsaceus, Momordica diocia* and *Momordica charantia var. muricata* was done and obtained result as shown in table 6.

Sr. no.	Name of plant crude drug	Alcohol soluble (%)		Water soluble (%)	
		Result	Standard value	Result	Standard value
1.	*Cucumis dipsaceus*	23.2	NLT 20 [4]	32.8	NLT 30 [4]
2.	*Momordica diocia*	16.8	NLT 6 [18]	36	NLT 21 [18]
3.	*Momordica charantia* var. *muricata*	14.4	NLT 8 [12]	30	NLT 28 [12]

Table 6: Extractive Value

Determination of ash value:

Ash value Determination for *Cucumis dipsaceus, Momordica diocia* and *Momordica charantia* var. *muricata* was done and obtained result as shown in table 7.

Sr. No	Name of Plant crude Drug	Total Ash Value %		Water soluble ash %		Acid insoluble ash %	
		Result	Standard	Result	Standard	Result	Standard
1.	*Cucumis dipsaceus*	5.5	NMT 6 [4]	1.5	5.2 [4]	2.95	NMT 4 [4]
2.	*Momordica diocia*	10.5	NMT 12 [18]	2	7 [18]	4.1	NMT 2.5 [18]
3.	*Momordica charantia var. muricata* (wild)	5.5	NMT 8.5 [12]	1.5	NIL	0.55	NMT 1 [7]

Table 7: ash value

Determination of Swelling Index:

Result: Swelling Index Determination for *Cucumis dipsaceus, Momordica diocia* and *Momordica charantia* var. *muricata* was done and obtained result as shown in table 8.

Sr. no.	Name of plant	Swelling index
1	*Cucumis dipsaceus*	1 ml
2	*Momordica diocia*	1 ml
3	*Moordica charantia muricata*	2 ml

Table 8: Swelling Index

Extraction of Phytoconstituents:

Extraction Of Phytoconstituents for *Cucumis dipsaceus, Momordica diocia* and *Momordica charantia var. muricata* was done and obtained result as shown in table 9.

Plant	**weight of sample (gm)**	**Solvent used**	**Qty. of Solvent**	**%Yield Value**
Cucumis dipsaceus	100	Petroleum ether	350ml	12
		Chloroform	350ml	16
		Methanol	350 ml	23.66
		Ethyl acetate	350ml	15
		Water	350ml	30
Momordica diocia	100	Petroleum ether	350ml	13
		Chloroform	350ml	10
		Methanol	350ml	20.26
		Ethyl acetate	350ml	11
		Water	350ml	36
Momordica charantia var. muricata	100	Petroleum ether	350ml	11
		Chloroform	350ml	14
		Methanol	350ml	16.2
		Ethyl acetate	350ml	28
		Water	350ml	28

Table 9: Extraction of Phytoconstituents

Preliminary Phytochemical Screening of Plant Extract:

Preliminary Phytochemical Screening of extract was done and obtained result as shown in table 10.

Used Abbreviations are: P.E.E=Petroleum ether extract, M.E=Methanol extract, C.E= Chloroform extract, E.A.E= Ethyl acetate extract, W.E= Water extract, - = Abscent, + = Present.

Constituents	*Momordica diocia*					*Momordica charantia var. muricata*				
	P.E.E	**M. E**	**C. E**	**E.A.E**	**W.E**	**P.E.E**	**M. E**	**C. E**	**E.A.E**	**W.E**
Carbohydrate: Fehling's Test	-	+	+	-	-	-	+	+	-	+
Molish Test	-	-	-	-	-	+	-	+	-	-
Barfoed's Test	-	-	-	-	-	-	-		-	-
Glycoside :Legal Test	-	-	-	-	-	-	-	+	-	+
Keller-Killani Test	-	-	-	+		+	+	+	-	-
Foam Test	-	-	-	-	+	-	-		-	
Borntrager's And Modified Borntrager's Test	-	-	-	-	-	-	-	+	-	+
Alkaloids: Dragendorff''s Test Mayer's Test Wagner Test Tannic Acid Test	 - - + -	 - - + -	 + + + -	 - + + +	 - - - -	 + - - +	 + - - -	 + - - -	 - - - -	 - + + -
Protein & amino acid Biuret test	-	-	-	-	-	-	-	+	-	-
Tannin & phenolic comp. Lead acetate test	+	+	-	+	-	+	+	-	+	+
Flavonoid: Shinoda test	-	-	+	-	-	-	+	+	+	-
Fixed Oil &Fat's Sudan Red Test	+	+	+	+	+	-		-	-	-
Steroid and triterpenoid Salkowski test	-	+	+	-	-	+	+	+	-	-
Waxes	-	-	-	-	-	-	-	-	-	-
Mucilage and gums	-	-	-	-	-	-	-	-	-	-

Constituents	*Cucumis dipsaceus*				
	P.E.E	**M.E**	**C.E**	**E.A.E**	**W.E**
Carbohydrates: Fehling Test	-	+	-	-	-
Molish Test	-	-	-	-	-
Barfoed's Test	-	-	-	-	-
Glycoside :Legal Test	-	+	-	-	-
Keller-Killani Test	-	-	-	-	
Foam Test	-	-	-	-	+
Borntrager's Test And Modified Borntrager's test	-	-	-	-	-
Alkaloids: Dragendorff''s Test Mayer's Test Wagner Test Tannic Acid Test	 - - - -	 - - + -	 - - - -	 - + + +	 - - - -
Protein & Amino Acid Xanthoprotein Test	+	-	-	-	-
Tannins & Phenolic Compounds Lead Acetate Test:	+	+	+	+	-
Flavonoid: Lead Acetate Test	-	+	+	-	-
Fixed Oil And Fats: Sudan Red Test:	-	+	-	+	+
Steroids & Triterpenoid: Salkowski Test	-	+	-	-	-
Waxes	-	-	-	-	-
Mucilage & Gums	-	-	-	-	-

Table 10: Preliminary Phytochemical Screening

Thin Layer Chromatography Study of Extract:

Thin Layer Chromatography Study of *Cucumis dipsaceus, Momordica diocia* and *Momordica charantia var. muricata* was done and obtained R_f-Value as shown in table 11, 12 and 13.

1. For *Cucumis dipsaceus* extract:

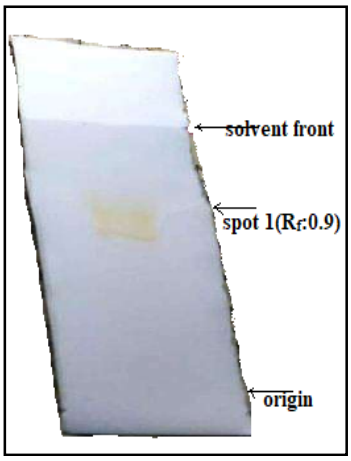

Fig. 12: TLC *Cucumis dipsaceus* extract (in day light)

No. of Spot	R_f-Value	
	Result (Day light)	**Standard** (As Per Wagner, Ref. No.11)
1.	0.90	0.9 (Cucurbitacin)

Table 11: Thin Layer Chromatography Study of *Cucumis dipsaceus* Extract

For *Momordica diocia* extract:

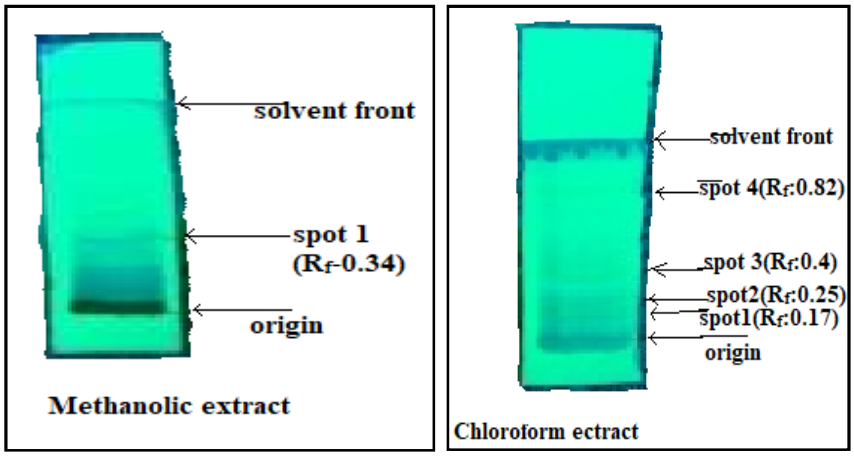

Fig. 13: TLC *Momordica diocia* extract (UV-365 nm)

No. Of Spot	R_f-Value		
	Result (UV-365 nm)		**Standard** (as per Ayurvedic Pharmacopoeia)
	Methanolic Ext.	Chloroform extract	
1.	0.34	0.17	0.16
2.	-	0.25	0.22(Cucurbitacin B glucoside)
3.	-	0.4	-
4.	-	0.82	0.9 (purified Cucurbitacin B)

Table 12: Thin Layer Chromatography Study of *Momordica diocia* extract

Momordica charantia var. muricata Linn:

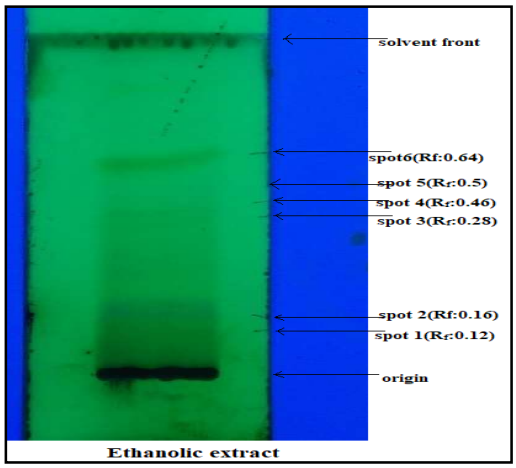

Fig. 14: In UV-Chamber in short light (254 nm).

No. Of Spot	R_f-Value	
	Result (UV-254 nm)	**Standard [12]**
1	0.12	-
2	0.16	0.17
3	0.28	0.23
4	0.46	0.46
5	0.5	-
6	0.64	0.67

Table 13: TLC Study of *Momordica charantia var. muricata* extract

Isolation of active chemical compound:

Result: isolation of Phytoconstituents Cucurbitacin and Charantin was done and percentage yield was found to be 0.71mg and 0.88 mg per gram.

Cucurbitacin Isolation:

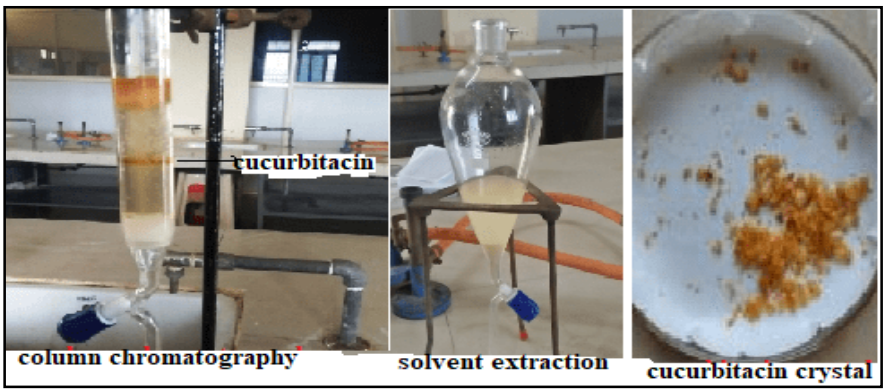

Fig. 15: Cucurbitacin Isolation

Charantin Isolation:

Fig. 16: Charantin Isolation

Identification of isolated compound:

TLC of isolated compound:

TLC of isolated compounds wad performed and obtain result as shown in table 14,15.

Cucurbitacin:

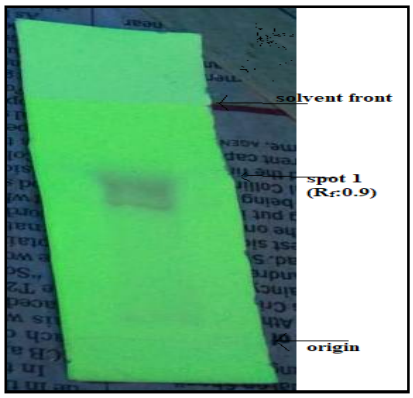

Fig. 17: Cucurbitacin TLC

No. Of Spot	R_f-Value	
	Result (day light)	**Standard** (As Per Wagner, Ref. No.11)
1	0.90	0.9

Table No.14 TLC of isolated compound Cucurbitacin

Charantin:

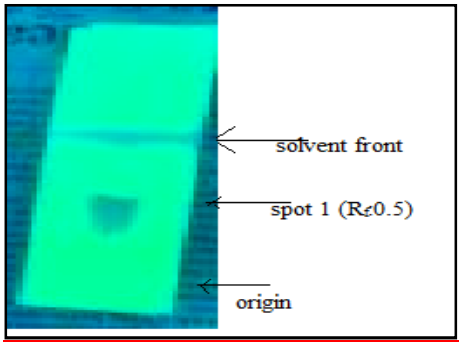

Fig.18: Charantin TLC

No. Of Spot	R_f-Value	
	Result (UV -254 nm)	**Standard** [15]
1.	0.5	0.5

Table 15: TLC of isolated compound Charantin

Melting point determination: method-capillary method

Melting point determination for isolated compound was done and shown in table 16.

Compound	**Melting point (˚C)**	
	Result	**Standard**
Cucurbitacin	180˚C	184˚C -186˚C [14]
Charantin	270˚C	268˚C [18]

Table No.16 Melting point determination

UV-spectroscopy of isolated compound:

The Wavelength of Maximum Absorption for Charantin Measured and obtained value was λ max 209 nm. Which near to standard λ max value 206 nm [17].

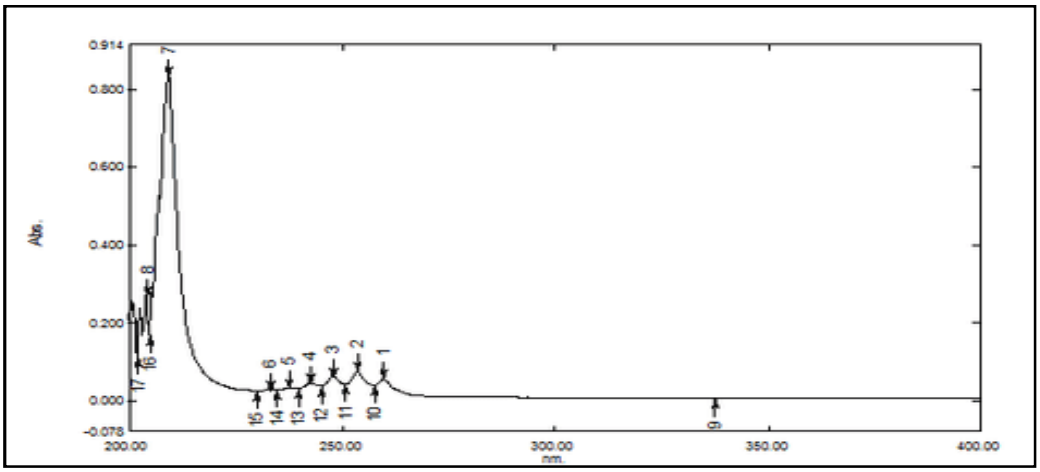

Fig.19: Wavelength of Maximum Absorption for Charantin

Conc.	Abs.
2ppm	0.047
4ppm	0.699
6ppm	1.232
8ppm	1.599
10ppm	1.958

IR-interpretation of isolated compound:

Charantin:

Graph:

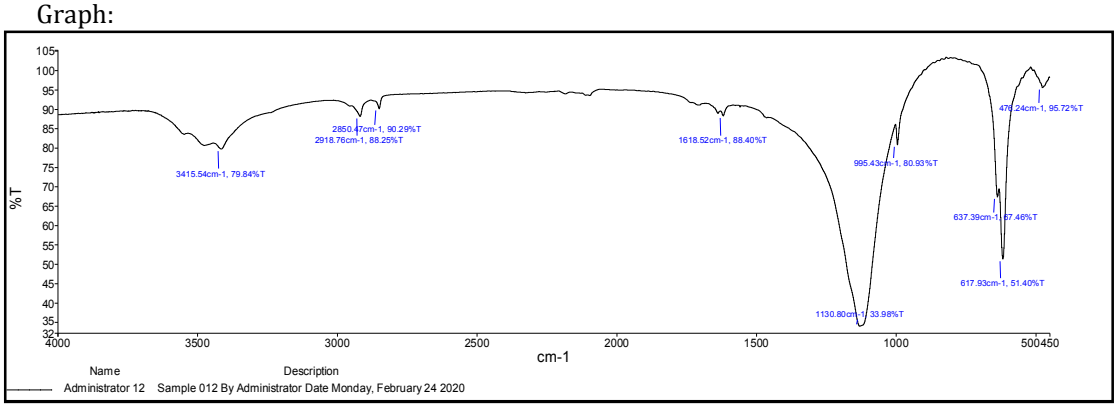

Fig.20: IR Spectra of Charantin

Obtained spectra of Charantin shows presence of conjugated C=C band at 1618cm^{-1} ,C-O stretching band 1130 cm^{-1}, >C=CH_2 (alkenes) at 995 cm $^{-1}$ and hydroxyl group band at 3415 cm^{-1} as shown in table 17.

Peak Name	Wave number (cm^{-1})	Functional group	Standard Reference
1.	3415.54	Free OH-stretching .(h-bonded alcohol, phenols)	[17]
2.	2918.76	C-H stretch (alkanes)	[8]
3.	2850.47	C-H stretch	
4.	1618.52	Unsaturation & C=C	[20]
5.	1130.8	C-O stretch (alcohol, carboxylic acids, esters, ethers)	[8]
6.	995.43	>C=CH_2 (alkene)	[20]

Table No.17 IR-interpretation of Charantin

Cucurbitacin :

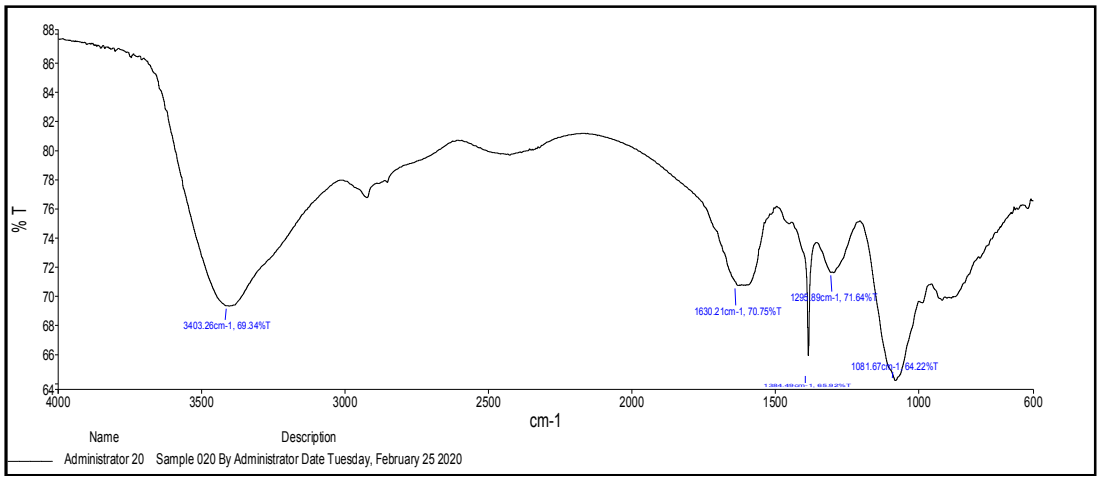

Fig.21: IR Spectra of Cucurbitacin

Obtained IR Spectra of Cucurbitacin Shows C-O in ester at 1081cm^{-1}. And C=C in alkene at 1630 cm^{-1}, C-H stretching band at 1384 cm^{-1}, -C-O- stretching in alcohol at 1295 cm^{-1} and Free OH-stretching h-bonded alcohol ,phenols at 3403 cm^{-1}. As shown in table 18.

Peak Name	Wave number (cm^{-1})	Functional group	Standard Reference
1.	3403.26	Free OH-stretching h-bonded alcohol ,phenols	[8]
2.	1630.21	C=C In alkenes	
3.	1384.49	C-H stretch	[14]
4.	1295.89	-C-O- stretching in alcohol	
5.	1081.67	C-O in ester	[8]

Table No.18 IR-interpretation of Cucurbitacin

CONCLUSION

The above study helps for standardization of crude drugs. The phytochemical analysis and thin layer chromatography helpful for Identity, Quality and Purity confirmation. The find information useful for therapeutic evaluation of plant extract. Further research can be carried out on isolated compound fraction for pharmacological evaluation to establish the medical significance of plants. The results will help as reference standards in quality control research carried over the above plant for isolation, purification and characterization of active principle and detailed pharmacological screening of each isolated compounds to find out probable mechanism of action. The outcome of above study is beneficial in different novel formulation development.

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