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Pharmacognostic study and preliminary phytochemical investigation of three samples of Karpasa(*Gossypium herbaceum* L.) root

M Tripathi[1], RLS Sikarwar[2], P K Shukla[3], AK Tiwari[1], N Dwivedi[1], S Tripathi[1], Ashwini A. Waoo[4]

[1]Arogyadham, Deendayal Research Institute, Chitrakoot, District Satna (M.P.) - 485 334
[2]Centre for Traditional Knowledge Research & Application, A.K.S. University, Satna (M.P.)-485 001
[3]Faculty of Pharmacy, Moradabad Institute of Technology (MIT), Moradabad (U.P.) 244001

[4]Department of Biotechnology, FLST, AKS University, Satna

Karpasa*(Gossypium herbaceum* L.)FamilyMalvaceaeis known forits economic and medicinalvalues. This shrub is calledKarpasa in Sanskrit and Kapasa in Hindi.It is used in the Ayurvedic system of medicine. All parts of the plant are used for the treatment of various disorders.This communication offers an in-depth pharmacognostic analysis of three samples of Karpasa root. The study encompasses macroscopic and microscopic examinations, powder microscopy, preliminary phytochemical assessments, physicochemical tests, heavy metal analyses, microbiological screening, HPTLC (High-Performance Thin Layer Chromatography) fingerprinting, and aflatoxin testing for the three samples.Physicochemical parameter tests were conducted, revealing average values across the three samples, including foreign matter at 2.63%, loss on drying at 105^0C at 4.36%, alcohol soluble extractive at 15.26%, hexane soluble extractive at 1.57%, water-soluble extractive at 17.41%, total ash 5.53%, acid insoluble ash 1.36%. The fingerprint profile of the methanolic extract was obtained using HPTLC (High-Performance Thin Layer Chromatography) with a toluene:ethyl acetate (7:3) mobile phase. 5% Methanolic-sulphuric acid derivatizing reagent was used to derivatize the TLC plate. Rf values and colour of the major spots were measured at 366 nm following derivatization at 366 nm and exposure to UV light. Aflatoxin (A1, B1, A2 & B2) test was performed and found absent, authenticated by comparing the Rf value and colour of the standard spots with the sample on the TLC plate.Following the execution of quantitative microbiological tests, several pathogens were shown to be absent, including total microbial count (TBC), yeast and mould, *Salmonella* sp./gm, *Pseudomonas aeruginosa*/gm, and *Staphylococcus aureus*/gm, while TBC was determined to be below WHO standards. After testing, heavy metal levels for Pb, Cd, As, and Hg were found to be within WHO guidelines. Set parameters can serve as benchmarks for quality assurance, plant identification in herbal ingredient formulations, and plant monograph creation. **Keywords:***Gossypium herbaceum*, HPTLC fingerprinting, Phyto-chemical investigation, Pharmacognostic, Microbiology,

Karpasa(*Gossypium herbaceum* L.)familyMalvaceaeis known by different names in different languages, *i.e.* Kapasa,Binaulain Hindi;Karpasa, Bona, Kapasia in English;Pambadana, Habb-ul-qutn in Urdu; Tulain Assamand Cotton plant in Bengali[1,2]. It is an annual or perennial shrub. It is cultivated in India as a dry land crop in most parts of India including the entire Deccan plateau and

Gujarat for its cottonfibres. Karpasa plant is 2 to 3.5 meters tall, leaves simple, petiolate, alternate, lamina ovate. Flowers- auxiliary, solitary on long erect pedicels bending back infruiting. Fruit an ovoid, acute 3-5 valved capsule, seeds upto 8 in each carpel, ovoid-subrotund with dull white cotton overlying a greyish, firmly adherent velvety coat.

Karpasa plants various parts like roots, bark, flowers and seeds are used to treat different types of human diseasessuch as Karnasrava (Otitis media), Pradara (Menorrhagia), Krushtha (leprosy),Pumsavana (procedure for male progeny) and preparation of Ayurvedic compound formulations. It contains yellow colour contents, (Cedral) 8%, Gossypol Dihydroxybenzoic acid, Salicylic acid, Betaine, Ceryl alcohol, Phytosterol[3,4,5]

Despite the plant's many reported medical benefits, no systematic pharmacognostic research on the root of the plant have been conducted to yet. The current study thus addresses the following areas: fluorescence research, microbiological screening, morphological analysis, anatomical evaluation, physicochemical testing, preliminary phytochemical screening, heavy metals testing, and High-Performance Thin Layer Chromatography.

Materials and methods

Collection of samples

The fresh plant root of Karpasa three samples were collected; one from the Arogyadhamcampus, Chitrakoot, Satna, Madhya Pradesh in March; the second from KVK, Majhgawan, Satna (M.P.) in March; and in March, a third sample was bought from the Karwi market in the Uttar Pradesh district of Chitrakoot. The plant was identified and authenticated.All Samples were identified and authenticated by Dr. R.L.S. Sikarwar, Senior Scientist, Deendayal Research Institute Chitrakoot. The voucher specimen (AD/AS/346/2019) was prepared as per standard procedure[5] and maintained in the herbarium of Arogyadham, Deendayal Research Institute, Chitrakoot, Satna (M.P.) for further reference. Anatomical studies employed fresh material, while physico-chemical, phytochemical, and High-Performance Thin Layer Chromatography fingerprint profiles were developed using shade-dried material that was ground into powder using an electric grinder.

Macroscopic study

Macroscopic or organoleptic characteristics ofKarpasa root likeappearance, colour, odour and taste wereevaluated.

Microscopic study

The fresh root section was cut by free handsectioning and numerous sections were examinedMicroscopically[6].Using Calliper Plus version 4.2 software, photos of the microscopical sections were taken using the Olympus Trinocular Research Microscope CX-21l equipped with a Digi-eye camera.

Powder microscopic study

After being ground into powder, the dried root passed entirely through an old sieve number 44, a 355 µm IS Sieve, and at least 50% through an old sieve number 85, a 180 µm IS Sieve. Approximately 2 grams of the powder were washed thoroughly with potable water, ensuring the material was not lost when the water was poured out.A small amount of glycerin was used to mount all of the Karpasa root's characteristics. One small quantity of sample was heated with chloral hydrate solution to remove any residue, and then it was cleaned, mounted, and treated with iodine solution for a few milligrams. Another small quantity of sample was stained with Sudan red solution and mounted in glycerin. All of the mounted

slides were viewed under a 40 x 10x trinocular research microscope.[7,8].

Physico-chemical parameters

Physical-chemical characteristics were calculated, including total ash value, acid insoluble ash value, water-soluble extractive value, hexane-soluble extractive value, and moisture content (loss on drying at 1050C).[9,10].

Preliminary phyto-chemical investigation

To check for the presence of phytoconstituents such as alkaloids, flavonoids, tannins, resins, carbohydrates, proteins, and saponins, preliminary phytochemical tests were conducted on ethanolic and water extract.[11,12,13].

High-Performance Thin Layer Chromatography (HPTLC) fingerprint profile

For High-performance thin layer chromatography, the powdered 5 gm of each sample (PRS, BRS & MRS) were extracted with 100 ml of ethanol overnight, filtered and concentrated. The application process involved spotting the extracted sample using a CamagLinomat -5 sample applicator and a 100 µl Hamilton syringe on a pre-coated silica-gel aluminium plate 60 F254 (5x10 cm with 0.2 mm layer thickness Merk Germany). Six-millimeter bands containing the samples were positioned 15 mm from the plate's bottom, 15 mm from its left edge, and 10 mm from its middle. A toluene, ethyl acetate, and formic acid (7.6: 2.5: 0.4 v/v)mobile phase was used to create the plates. A glass chamber that was equilibrated with a mobile phase was used to conduct a linear ascending development in a 10x10cm twin. The mobile phase was allowed to saturate the chamber for 30 minutes at room temperature. The chromatogram was developed over a length of 8 cm using 20 ml of the mobile phase. After development, the Thin Layer Chromatography plate was dried using a hot air oven.

The camera photo documentation system CamagReprostar 3 was used to record the peak area for samples and standards. with Win Cat software, the spot was visualized both before and after derivatization (with 5% Methanolic - Sulfuric Acid Reagent) under UV light, and Rf values were recorded.[14,15,16,17].

Test for aflatoxins

Aflatoxins are highly dangerous for the human body. This test is provided to detect the possible presence of aflatoxins B_1, B_2, G_1 and G_2 in any material of plant origin. Three samples of Karpasa root were checked for mycotoxin, i.e. Aflatoxin with standard markers B_1, B_2, G_1 and G_2[18].

Microbiological limit tests

Microbial limit tests are used to identify specific microbial species in medicinal compounds and to estimate the quantity of viable aerobic microorganisms present. The following tests were carried out as per[10]to determine the microbial load in three samples of Karpasa root powder.

Enumeration of *Staphylococcus aureus*/gm
Enumeration of *Salmonella sp.*/gm
Enumeration of *Pseudomonasaeruginosa*/gm
Enumeration of *Escherichia coli*
Determination of total microbial count (TBC)
Determination of Yeast &Mould

Specific agar media and enrichment media from Himedia, Pvt. Ltd. in Mumbai were used to determine the results of the microbiological testing.

Result

Macroscopic characters

Karpasa root long woody cylindrical tap root with few lateral roots and branches. Surface- rough, shows tangentially, lenticels, and scars left by lateral rootlets. Fracture outer is short, and the inner fibrous, exposing the inner cream colourof the wood; the colouris yellowish brown externally, tastes slightly bitter, odour characteristics(Fig.1a&1b).

Microscopic characters

TS of Karpasa root is circular in outline and shows an outer narrow cork and parenchymatous cortex embedded with idioblasts of cluster crystals of calcium oxalate; phloem is broader and occupies the major portion of bark and traversed by several narrow radial strips of fibres alternating with 2 to 4 seriate medullary rays in continuation with xylem rays; cambium is distinct, xylem composed of vessels, tracheid's and fibres(Fig. 2a, 2b &2c).

Powder microscopic characters

Karpasa root powder colour is whitish brown, tastes no characteristics and is odour astringent.

Under microscope powder shows cork cells in surface view, cork cells in sectional view, parenchymatous cells, simple pitted vessels, group of stone cells, fragments of radially-longitudinally cut medullary rays crossing the fibre, tangential-longitudinal section showing medullary rays, fibres, and row of phloem parenchyma, cluster crystals of calcium oxalate, prismatic crystals of calcium oxalate, fibres, fragments of medullary rays containing crystals and starch grains (Fig. 3)

Physico-chemical analysis

Physicochemical parameters such as extractive values are useful for assessing whether a drug is exhausted or adulterated, while ash values provide insight into the drug's inorganic composition, including earthy matter and other impurities.Three samples of Karpasa root powder physico-chemical resultsare given in (Table 1)

Heavy metals tests

Heavy metal elements (Pb, Cd, As and Hg) tests were performed and found under limits as per guideline WHO and results are given in (Table 2).

Microbiological limit tests

The microbiological profile of the Karpasa root powder was found satisfactory under limits as per guideline WHO. Results are given in (Table 3).

Preliminary phyto-chemical investigation

The extracts obtained in ethyl alcohol and water were tested for qualitative phyto-constituents. Protein, tannin, saponin, alkaloids, and flavonoids were detected during screening. *HPTLC fingerprint profile*

The ethanolic extract of three locations of the Karpasa root sample extract was studied using high-performance thin layer chromatography (HPTLC) and applied to a precoated TLC plate. Using a solvent system of toluene, ethyl acetate, and formic acid (7.6: 2.5: 0.4 v/v) at a distance of 8 cm, 6 µl of the test solution was applied in 8 mm bands to the plate. Dry the developed plate at room temperature and examined. Derivatized the plate using 5% *Methanolic-sulphuricacid* reagent and heating at 105^0C till the bands were visible.The Rf values and colors of major spots were documented both before and after derivatization, at 366 nm and under UV light.The chromatogram profile and corresponding Rf values are provided (Fig. 4 & Table 4).

Test for Aflatoxins

Aflatoxins (B_1; B_2; G_1& G_2) study of the ethanolic extract three spots of the Karpasaroot sample extract and four standards of Aflatoxins (B_1; B_2; G_1& G_2) applied in precoated TLC plate.10 µl of the test solution were applied as 8 mm bands and the plate was developed in a solvent system consisting of toluene, ethyl acetate, and formic acid (7:2.5:0.5) to a distance of 8 cm.

The developed plate was dried at room temperature and examined under 366 nm light. Major spot Rf values and colors were recorded at 366 nm. The chromatogram profile and Rf values are presented (Fig. 5& Table5).

Discussion

The macroscopic, microscopic and powder microscopic distinguished characters have been established to identify the *Karpasaroot*. The pharmacognostic and physicochemical parameters can be used for checking the adulteration and purity of this drug. HPTLC fingerprint profile helps in the identification of various phytochemical constituents present in the crude drug thereby substantiating and authenticating of crude drug. The TLC profile also helps to identify and isolate important phyto-constituents. The Aflatoxins were absent in the Karpasa root samples. Heavy metal elements are found under limits as per guidelines WHO and microbial limits test of the Karpasaroot were found satisfactory. Total microbial plate count (TBC), Yeast &Moulds counts were reported less than the limit as suggested by WHO and pathogenic bacteria i.e.,*Staphylococcus aureus*, *Salmonella sp*., *Pseudomonasaeruginosa* and*Escherichiacoli* were found to be absent. All findings indicate indicating samples are genuine and free from any adulterations. These findings could be helpful in the identification and authentication of Karpasa root.

Conclusion

Due to the side effects of modern medicines on human health, the importance and uses of herbal medicines are increasing day by day all over the world. Because the plants have natural chemicals which do not have any adverse side effects on human health. Herbal medicines,however, suffer from a lack of standardization parameters and quality control. Hence the standardization and quality control of herbal drugsare very important. Karpasa (*Gossypium herbaceum*L.) is one of the most important plants of India and its different parts such as root, bark, flowers and seeds are used to treat different types of human ailments and diseases such as otitis media, menorrhagia, leprosy, procedure for male progeny and preparation of Ayurvedic compound formulations like Karpasasthitaila etc. Due to its wide therapeutic importance, it is worthwhile to standardize it for use as a drug. It contains yellow colour contents, (cidral) 8%, Gossypol Dihydroxybenzoic acid, Salicylic acid, Betaine, Ceryl alcohol and Phytosterol.

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Conflict of interest

The authors declare no conflict of interest.

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Table1-Physico-chemical analysis of Karpasa root

S. N.	Name of Parameters	Results			Average value
		Arogyadham campus, Chitrakoot	KVK, Majhgawan	Karwi Market	
1	Foreign Matter	2.5%	2.5%	2.9%	2.63%
2	LOD at 105 ^{0}C (%w/w)	4.52%	4.47%	4.09%	4.36%
3	Alcohol soluble extractive value(% w/w)	15.33%	15.10%	15.36%	15.26%
4	Hexane soluble extractive value(% w/w)	1.31%	1.70%	1.71%	1.57%
5	Water soluble extractive value(% w/w)	17.29%	17.85%	17.11%	17.41%
6	Total ash value (% w/w)	5.7%	5.40%	5.5%	5.53%
7	Acid in soluble ash value (% w/w)	1.3%	1.4%	1.4%	1.36%

Table2- Determination of heavy metals of Karpasa root

S. N.	Name of Tests	Results			WHO/ API Limits
		Arogyadham campus, Chitrakoot	KVK, Majhgawan	Karwi Market	
1	Lead (Pb)	0.3738ppm	0.3738ppm	0.3770ppm	10 ppm
2	Cadmium (Cd)	0.0406 ppm	0.0451ppm	0.0405ppm	0.3 ppm
3	Arsenic (As)	5.8750ppb	5.3567ppb	5.5794 ppb	03 ppm
4	Mercury (Hg)	2.5875ppb	2.6712 ppb	2.4478ppb	01 ppm

Table-3 Microbial limit test of Karpasa root

Rf Value	Before Derivation	After Derivation	
	366nm	366nm	UV Light
$R_{f\,1}$	0.06 (red)	0.06 (brown)	0.06 (brown)
$R_{f\,2}$	0.12 (pink)	10. (sky blue)	0.70 (sky blue)
$R_{f\,3}$	0.20 (blue)	0.22 (sky blue)	0. 80 (brown)

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$R_{f\,4}$	0.28 (blue)	0.28 (blue)	-
$R_{f\,5}$	0.40 (pink)	0.40 (brown)	-
$R_{f\,6}$	0.50(pink)	0.50 (brown)	-
$R_{f\,7}$	0.60 (pink)	0.56 (brown)	-
$R_{f\,8}$	0.70 (pink)	0.58 (blue)	-
$R_{f\,9}$	0.78 (blue)	0.60 (brown)	-
$R_{f\,10}$	0.80 (red)	0.70 (white)	-
$R_{f\,11}$	0.90 (red)	0.90 (brown)	-

Table-4 R_f value of HPTLC fingerprint of Karpasa root

S.N.	Test	Arogyadham campus, Chitrakoot	KVK, Majhgawan	Karwi Market	Permissible limits as per WHO/ API
1	*Staphylococcus aureus /g*	Absent	Absent	Absent	Absent
2	*Salmonella sp. /g*	Absent	Absent	Absent	Absent
3	*Pseudomonas aeruginosa/*	Absent	Absent	Absent	Absent
4	*E.coli*	Absent	Absent	Absent	Absent
5	Total microbial plate count(TPC)	275 cfu/g	278 cfu/g	280 cfu/g	10^5 / cfu/g
6	Total Yeast and Mould	100 cfu/g	110 cfu/g	105 cfu/g	10^3 / cfu/g

Table 5: R_f values in the test solution for Aflatoxin in *Karpas root* powderat 366 nm

R_f Values	Standard Aflatoxin				Results		
	B_1	G_1	B_2	G_2	Arogyadham campus, Chitrakoot	KVK, Majhgawan	Karwi Market
$R_{f\,1*}$	0.51	-	-	-	Not Seen	Not Seen	Not Seen

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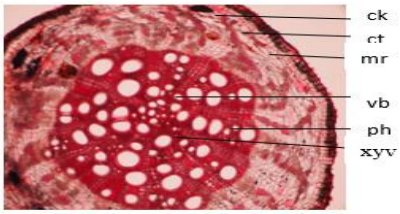
$R_{f\ 2}$ *	-	0.40	-	-	Not Seen	Not Seen	Not Seen
$R_{f\ 3}$ *	-	-	0.44	-	Not Seen	Not Seen	Not Seen
$R_{f\ 4}$ *	-	-	-	0.36	Not Seen	Not Seen	Not Seen

* Fluorescent colour

Fig. 1a

Fig. 1b

Fig.1- 1a karpasa plant, 1b pieces of dried root of karpasa



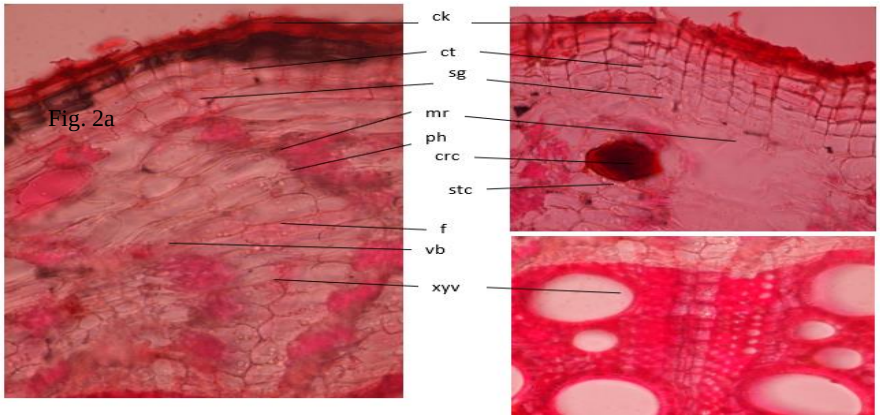


Fig. 2a

Fig. 2b

Fig. 2c

Fig. 2- 2a diagrammatic TS of root, 2b TS of karpasa root, 2c detailed TS of root

Abbreviation-ck, cork cells; ct, cortex; sg, starch grains; mr, medullary rays, ph, phloem, crc, cluster crystals of calcium oxalate; stc, stone cells; f, fibre; vb, vascular bundle; xyv, xylem vessels

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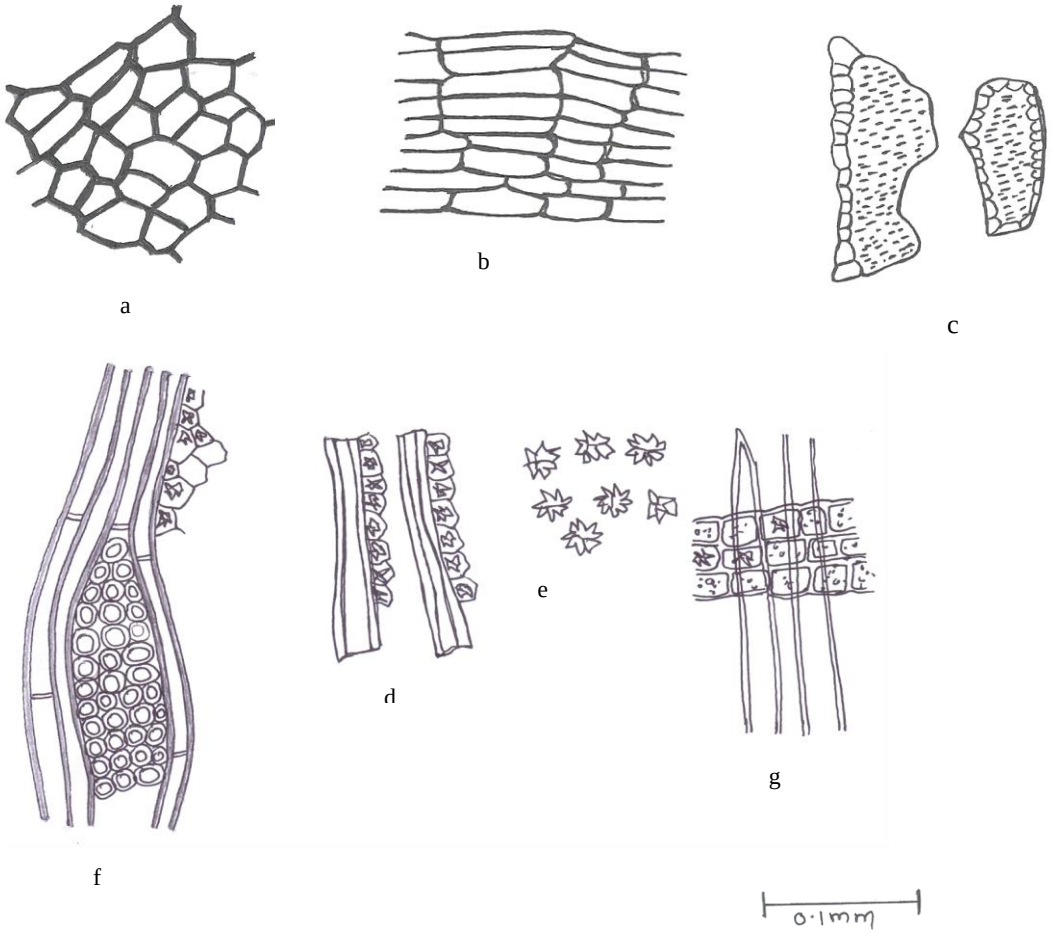

Fig. 3-Powder microscopic characters of Karpasa root (a) Cork cells in surface view, (b) Cork cells in sectional view, (c) Simple pitted vessels, (d) Crystals fibres, (e) Cluster crystals of calcium oxalate (f) Tangential-longitudinal section showing medullary rays, fibres, and row of phloem parenchyma (g) Fragments of radially-longitudinally cut medullary rays crossing the fibre

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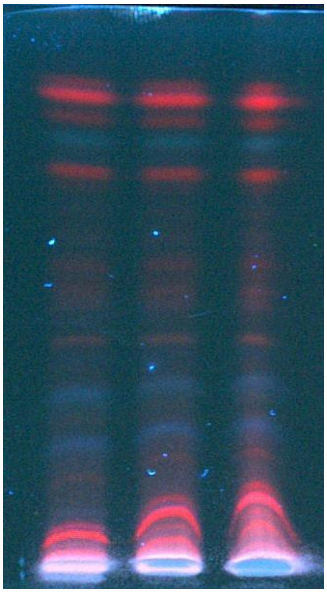
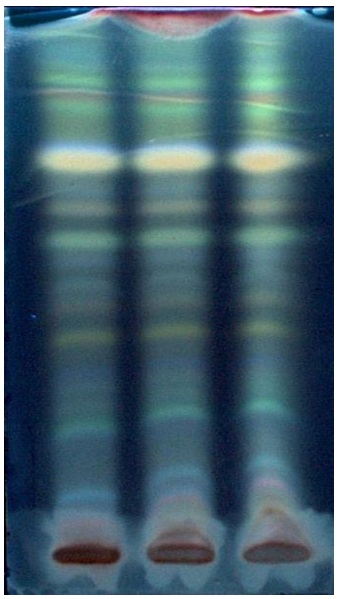


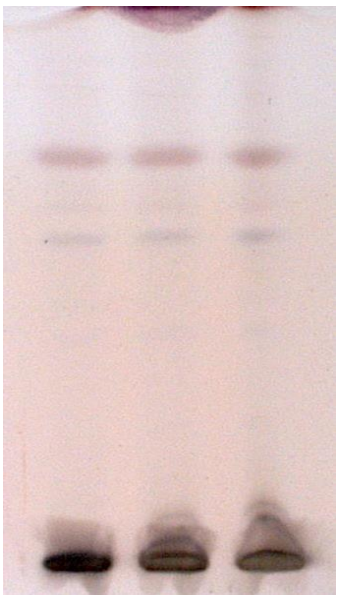
A B C

Fig. 4A



A B C

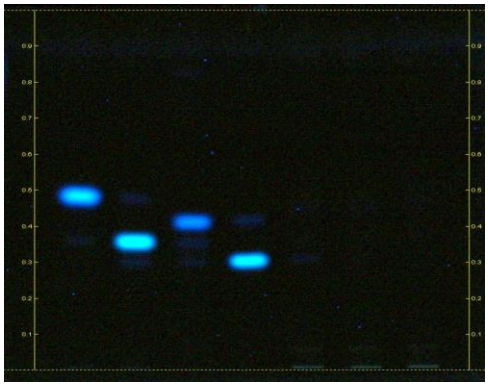
Fig. 4B



A B C

Fig. 4C

Fig. 4- HPTLC fingerprints profile of Karpasa root, where Fig. 4A at 366nm before derivatization; Fig. 13B at 366nm after derivatization; Fig. 13C at UV light after derivatization. Where Tracks A=Sample Arogyadham campus, Chitrakoot ; trackB = Sample KVK, Majhgawan; track C= Sample Karwi Market



B_1 G_1 B_2 G_2 S1 S2 S3

Fig. 5- Aflatoxin test of Karpasa root at 366 nm
Where Tracks: B_1, G_1, B_2, G_2 standard markers ; and track S_1 =Sample Arogyadham campus, Chitrakoot ; track S_2 =Sample KVK, Majhgawan; track S3= Sample Karwi Market