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Standardization of Dasana-Samasarkara Curna -A Polyherbal Ayurvedic Formulation

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Abstract

India is vast repository of medicinal plants that are used in traditional medical treatments. Herbal medicines as the major remedy in traditional system of medicine have been used in medical practices since antiquity. Medicinal plants play an important role in the development of potent therapeutic agents. To keep this view in mind, a polyherbal Dasana-Samasarkara Curna is formulated in house, which is very effective in Mukharoga (disease of mouth), Dantaroga (disease of tooth). It formulated using ten single drugs viz. *Zingiber officinale* Roxb.(rhizome), *Terminalia chebula* Retz.(fruit), *Cyperus rotundus* Linn.(rhizome), *Acacia catechu* (Linn.f.) Willd (heart wood), *Cinnamomum camphora* (L.) Nees & Ebrm & ocimum kilimandscharicum guerke(sub.Ex.), *Areca catechu* Linn.(seed bhasma), *Piper nigrum* Linn.(fruit), *Syzygium aromaticum* (Linn.) Merr.& M. Perry (flower), *Cinnamomum zeylanicum* Blume(stem bark), Kathini (khatikā) Kaolinite (Clay). All the ingredients and curna were analyzed in order to assess the authenticity of the drugs based on ayurvedic requirement following a series of powder microscopy, physico-chemical, HPTLC finger printing and microbiological parameters. The obtained values/ranges can adopted to pay down new pharmacopoeial standards, to be followed for traditional preparation of Dasana-Samasarkara Curna.

Key-Words: Standardization, Dasana-Samasarkara Curna, Powder microscopy, HPTLC, Physico-chemical

Introduction

Herbs had been used by all cultures throughout history. It was an integral part of the development of modern civilization. Ayurveda like traditional medical science is the one of the most ancient medical science of the world. Basic feature of Ayurveda is its holistic approach to treat human beings as a whole and it restore harmony among the human beings, plants and environment. By knowing the standards of a medicine following qualitative and quantitative parameters and method followed in single and formulated drug can improve the efficacy and efficiency of drug as well as confidence of doctor and patient.

Goal of standardization is to assess the authenticity of the drug based on the above principal. To keep this view in mind, a polyherbal Dasana-Samasarkara Curna is formulated in house, which is very effective in Mukharoga (disease of mouth), Dantaroga (disease of tooth). It formulated using ten single drugs viz. *Zingiber officinale* Roxb.(rhizome), *Terminalia chebula* Retz.(fruit), *Cyperus rotundus* Linn.(rhizome), *Acacia catechu* (Linn.f.) Willd (heart wood), *Cinnamomum camphora* (L.) Nees & Ebrm & ocimum kilimandscharicum guerke (sub.Ex.), *Areca catechu* Linn.(seed bhasma), *Piper nigrum* Linn.(fruit), *Syzygium aromaticum* (Linn.) Merr.& M. Perry

(flower), *Cinnamomum zeylanicum* Blume(stem bark), Kathini (khatikā) Kaolinite (Clay)¹.

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Present study described the Standardization of Dasana-Samasarkara Curna-A Polyherbal Ayurvedic Formulation and its single ingredients². Both are useful in Siddha, Unani and Ayurveda as single or in combination with other drugs. Hence the purpose of standardization of raw drugs and formulation is obviously to ensure the therapeutic efficacy of the drug³.

Material and Methods

Method of Preparation of the Curna

All the ingredients of the Dasana-Samasarkara Curna were used in pharmacopoeial quantity¹. These were washed, dried and ground individually passed through 180µm separately then weighed separately, mixed in specified ratio and passed through 355µm to obtain a homogenous blend. Stored in air-tight container to protect from light and moisture. Three different samples of Dasana-Samasarkara Curna, two samples were prepared at research laboratory Ayurveda Sadan, Chitrakoot Batch-A and Batch-B where Batch-C was prepared by Chitrakoot Ras-sahalsa Pharmacy.

Method of Slide Preparation for Microscopic Characters

For microscopic analysis⁴ about 10 gm of formulated curna in a small beaker, added water, stir thoroughly and pass through 150 μm IS Sieve (old sieve number 100) to remove *kathini*; repeat the process of washing, collect the plant residue on the sieve and wash thoroughly with potable water repeatedly without loss of plant material; mount a small portion in *glycerine*; warm a few

portion in *glycerine*; warm a few mg with *chloral hydrate* solution, treat a few mg with *iodine* in *potassium iodide* solution and mount in *glycerine*; treat a few mg with *ferric chloride* solution and mount in *glycerine*; heat a few mg in 2 percent aqueous *potassium hydroxide*, wash in water and mount in *glycerine*. Take about 0.5 g of sample and add 50 percent conc. *Nitric acid* in a test tube and warm over water bath till brown fumes appear; add a few crystals of *potassium chlorate* cool and wash with water thoroughly and mount a small portion in *glycerin*. Observed the characteristics in the various mounts.

Physicochemical Tests

organoleptic characters, particle size and physicochemical analysis of all the samples were carried out. Quantitative analysis for loss on drying at 105°C, alcohol soluble extractive, water soluble extractive, total ash, acid insoluble ash and pH of filtrate of 10% w/v aqueous solution were checked in triplicate.⁵⁻⁷

High Performance Thin Layer Chromatography (HPTLC) Profile

For HPTLC, 2gm of each sample was extracted with 25 ml of methanol on boiling water bath for 25 minute consecutively of 3 times using fresh portion of 25 ml methanol, filtrate and concentrated. TLC of extracts of all the samples was carried out on Silica Gel 60 F₂₅₄ precoated plates (0.2 mm thickness; from Merck India Limited Mumbai). An applicator from Camag Linomat-5 (Camag Switzerland 140443) was used for band application and photo documentation unit (Camag Reprostar-3: 140604) was used for documentation of chromatographic fingerprints. The mobile phase used *Toluene: Ethyl acetate* (7:3). The plate was developed over a distance of 9 cm in a saturated development chamber (Twin trough chamber (10 x 10 cm with SS lid, and visualized under visible light, 254nm and 366nm. After spraying with 5% methanolic sulphuric acid followed by heating at 110°C for 5-10 minute⁸.

Microbiological Tests

PDA, Mac-conkey broth, XLD, plate count agar, Cetrimide agar and *Staphylococcus aureus* enriched agar media were used for the isolation of the Yeas t& Moulds, *E.coli*, *Salmonella sp.*, TBC, *Staphylococcus*

sp. & *Pseudomonas sp.* To perform this test different proportion of different media were dissolved in different flasks containing distilled water. All the media were prepared and autoclaved at 15 lb/in² pressure at 121°C for 15-20 minutes. After autoclaving media were poured into Petriplates and allowed to solidify. Plates were prepared & marked for different isolated. Culture suspension was prepared in 1 gm of test sample in 100 ml. distilled water. 0.1 ml suspension was spreaded on the plates with the help of spreader. All plates were prepared, inoculated & incubated at 26-28°C for 24-28 hours. After incubation period, the observations were taken for presence or absence of the colonies⁹. The concentration of different microorganisms and media used were given.

Results and Discussion

Daśan Samskāra cūrṇa samples from 2 different manufacturers, Batch -A, Batch-B, Batch-C, were subjected to analysis as above. All samples were grayish white powder with a characteristics spicy odour and taste. The powder completely passes through 355 μm IS Sieve and not less than 50% through 180 μm IS Sieve. The result of loss on drying at 105°C 7%, total ash content 44%, acid insoluble ash 16%, alcohol soluble extractive values 6%, water soluble extractive value 5%, and pH 5.45-6 were calculated and are given in Table-1.1 to 1.3. Microscopic examinations were also carried out to see presence of the different ingredients in all 3 samples of Daśan Samskāra cūrṇa (Fig. 1-2). Parenchymatous cells containing oleo-resin, oval to round starch grains not less than 15 μ to 30 μ and several up to 70 μ with hilum eccentric, lamellae distinct, pitted, septate fibres with indentations on its walls (*Śunthī*); thick walled elongated sclereids of various shapes and sizes, pitted parenchymatous cells, polygonal cells with beaded walls and several showing thin septum, fibres with peg like growth near tips, lumen broad and criss- cross layer of fibre groups (*Haritakī*); fibre sclereids from scale leaves in packed rows, beak shaped starch grains 6 μ to 28 μ , narrow vessel units with scalariform thickening (*Mustā*); crystal fibres associated with phloem fibres, longitudinally cut medullary rays surrounding with phloem parenchyma filled with starch grains, prisms and yellow contents, xylem fibres with brown contents (*Khadira*); hypodermal parenchyma interspersed with stone cells, thick walled polygonal stone cells from testa (*Marica*); small rounded triangular tetrahedral pollen grains 15 μ -20 μ , elongated parenchymatous cells containing rosette crystals of calcium-oxalate, fragments of epidermal cells with anomocytic stomata in surface view, shizolysigenous oil cells (*Davapuspa*); parenchymatous cells containing minute acicular

crystals of calcium-oxalate, fibres with narrow lumen, 250 μ -600 μ long and 25 μ -45 μ broad, cells from cortex with thick inner periclinal walls as well as cells with three thick inner walls (Tvaka). Plate-1 (Fig.1-5 and Plate-2, Fig. 6-7)

TLC of methanolic extracts of all the samples were carried out on Silica gel plates (Plate-3, Fig. 1-4). Several bands were observed, some of which were

specific to the individual ingredients. These bands were chosen to identify the presence or absent of the individual ingredients in each of the three samples. It was observed that all three identifying bands were present in all of Curna samples thereby indicating the presence of all the 10 ingredients in each of them. Table 2.1- 2.4 enlist identifying bands of the individual ingredients along with their Rf values.

Table 1.1: Physico-chemical parameters of Daśan Samskāra cūrṇa and used single raw drugs

Name of Curna with ingredients	LOD (%w/w)	Total ash (% w/w)	AI ash (% w/w)	ASE (% w/w)	WSE (% w/w)
Batch -A	6.53	43.90	15.11	5.3	7.2
Batch- B	6.50	43.54	15.15	5.1	6.7
Batch- C	6.49	43.54	15.16	5.1	6.9
Sunthi	8.68	6	0.97	3	10
Haritakī	6.09	3.24	3.28	48.11	63.78
Musta	7.54	2.87	1.86	9.78	17.40
Khadira	8.49	1.19	0.12	3.83	6.16
Marica	6.95	4.28	0.43	7.14	11.46
Lavanga	3.22	5.36	0.66	14.16	28.06
Tvak	8.15	2.9	1.8	4.77	4.73

Table 1.2: Physicochemical properties of Karpura

Name of tests	Result
Identification	Volatilize at ordinary temperature and readily burns with a smoky flame
Melting range	175 ⁰ to 177 ⁰
Specific optical rotation	+41 ⁰ , +43 ⁰
Non-volatile matter	0.04
Camphor	0.97

Table 1.3: Physicochemical properties of khatika

Test	Physical properties
Nature	Lumps with greasy feel and earthy odour
Colour	Whitish, occasionally tinted
Streak	White
Cleavage	Not observable
Fracture	None
Transparency	Translucent
Silica	45%
Alumina	54%
Distinctive Properties	
Small quantity of <i>Khatika</i> powder stained with <i>alcoholic methylene blue solution</i>	Most particles are stained a deep blue
Small quantity of <i>Khatika</i> powder stained with <i>Safranin solution</i>	None or very few particles are coloured red
Small quantity of <i>Khatika</i> powder added few drops of <i>hydrochloric acid</i>	No gas or bubbles evolved

Table 2.1: R_f values in TLC finger prints at 366nm

R _f values	366 nm		
	Batch-A	Batch-B	Batch-C
R _f 1(blue)	0.08	0.08	0.08
R _f 2 (brown)	0.13	0.13	0.13
R _f 3 (blue)	0.26	0.26	0.26
R _f 4 (red)	0.30	0.30	0.30
R _f 5 (blue)	0.34	0.34	0.34
R _f 6 (blue)	0.39	0.39	0.39
R _f 7 (brown)	0.61	0.61	0.61
R _f 8 (blue)	0.82	0.82	0.82
R _f 9 (red)	0.89	0.89	0.89

Table 2.2: R_f values in TLC finger prints at 366nm after spraying

R _f values	366nm after spraying		
	Batch-A	Batch-B	Batch-A
R _f 1 (blue)	0.08	0.08	0.08
R _f 2 (blue)	0.15	0.15	0.15
R _f 3 (blue)	0.22	0.22	0.22
R _f 4 (blue)	0.28	0.28	0.28
R _f 5 (lightyellow)	0.43	0.43	0.43
R _f 6 (blue)	0.50	0.50	0.50
R _f 7 (yellow)	0.57	0.57	0.57
R _f 8 (yellow)	0.60	0.60	0.60
R _f 9 (grey)	0.78	0.78	0.78

Table 2.3: R_f values in TLC finger prints at Visible light after spraying

R _f values	Visible light after spraying		
	Batch-A	Batch-B	Batch-A
R _f 1(black)	0.08	0.08	0.08
R _f 2(brown)	0.15	0.15	0.15
R _f 3(brown)	0.39	0.39	0.39
R _f 4(brown)	0.50	0.50	0.50
R _f 5(brown)	0.61	0.61	0.61
R _f 6(red)	0.78	0.78	0.78
R _f 7(black)	0.86	0.86	0.86
R _f 8(brown)	0.94	0.94	0.94

Table 2.4: R_f values in TLC finger prints at 254nm

R _f values	254 nm		
	Batch-A	Batch-B	Batch-A
R _f 1(black)	0.07	0.07	0.07
R _f 2 (black)	0.10	0.10	0.10
R _f 3 (black)	0.39	0.39	0.39
R _f 4 (black)	0.50	0.50	0.50
R _f 5 (black)	0.54	0.54	0.54
R _f 6 (black)	0.70	0.70	0.70
R _f 7 (black)	0.82	0.82	0.82

Conclusion

Present studies, it can be calculated that the microscopical characteristics and the distinguishing band in the HPTLC profiles are very important for monitoring the quality of the Curna formulation as well as for establishing whether all the required ingredients are present in them. Also standardization and development for reliable quality protocols for Ayurvedic formulations are important for keeping a check on batch to batch variations. Hence, the physicochemical parameters, quantitative analysis, HPTLC fingerprinting profiles and the microscopic characteristics together may be used for quality evaluation and the standardization of compound formulations and maintaining their quality, purity and efficacy.

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Plate-1: Powder Characteristics of Dasana-Samasarkara Curna

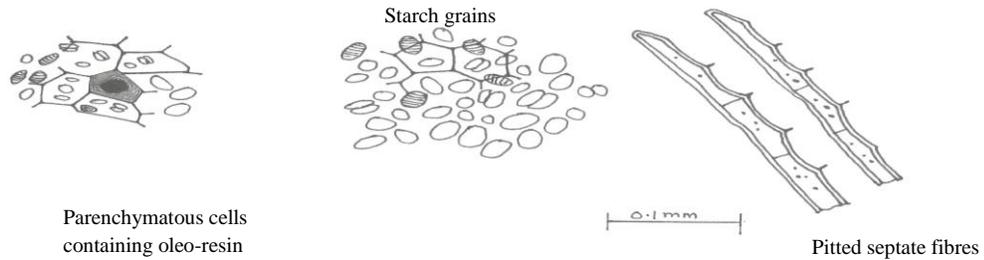


Fig. 1: Śuṅṭhī

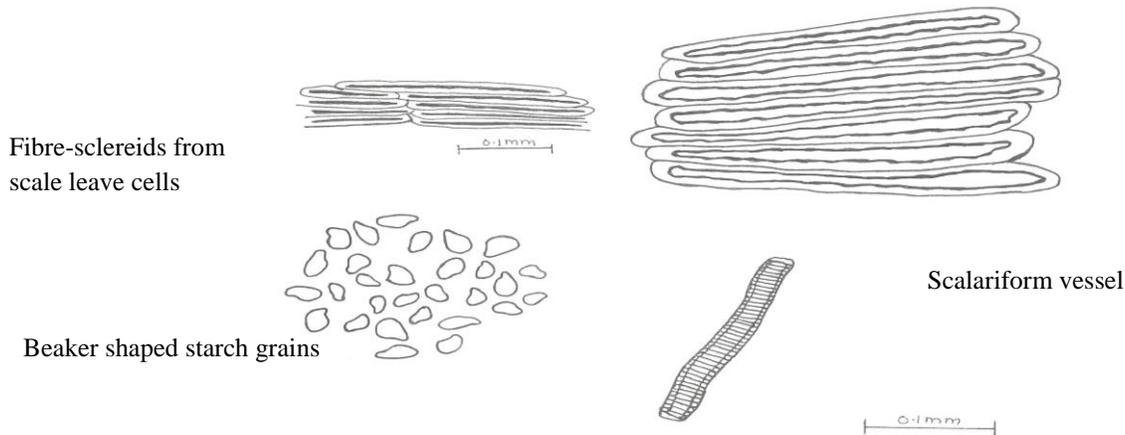


Fig.3: Mustā

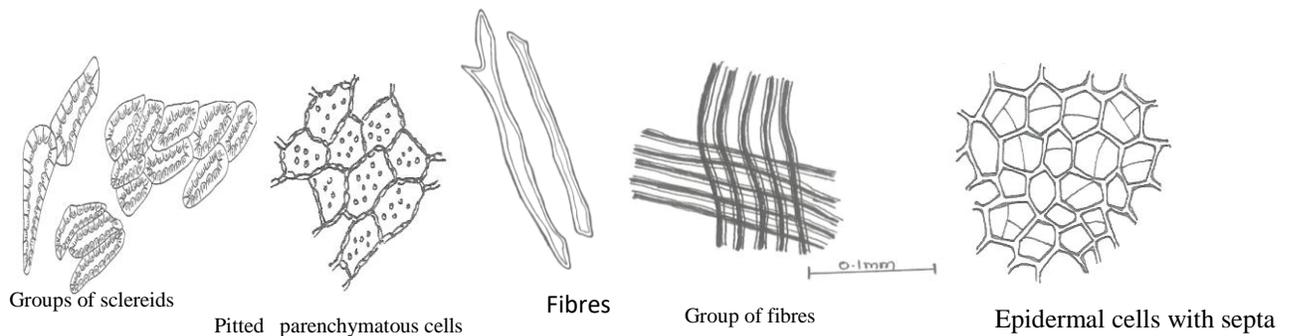


Fig. 2: Haritakī

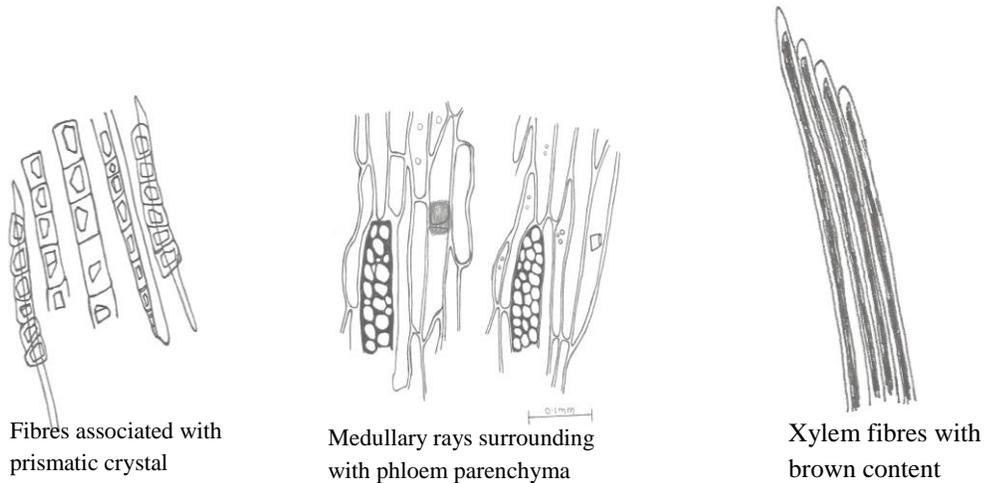


Fig. 4: Khadira

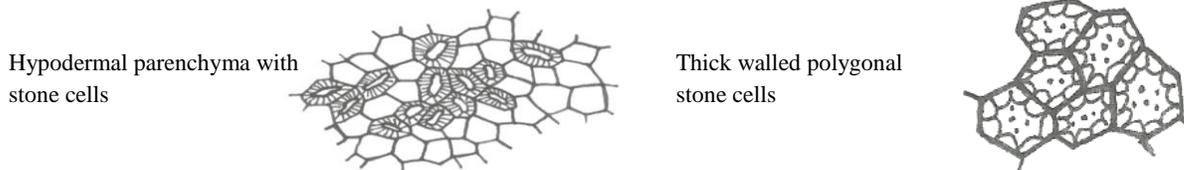


Fig. 5: Marica

Plate 2: Powder characteristics of Daśan Samskāra cūrṇa

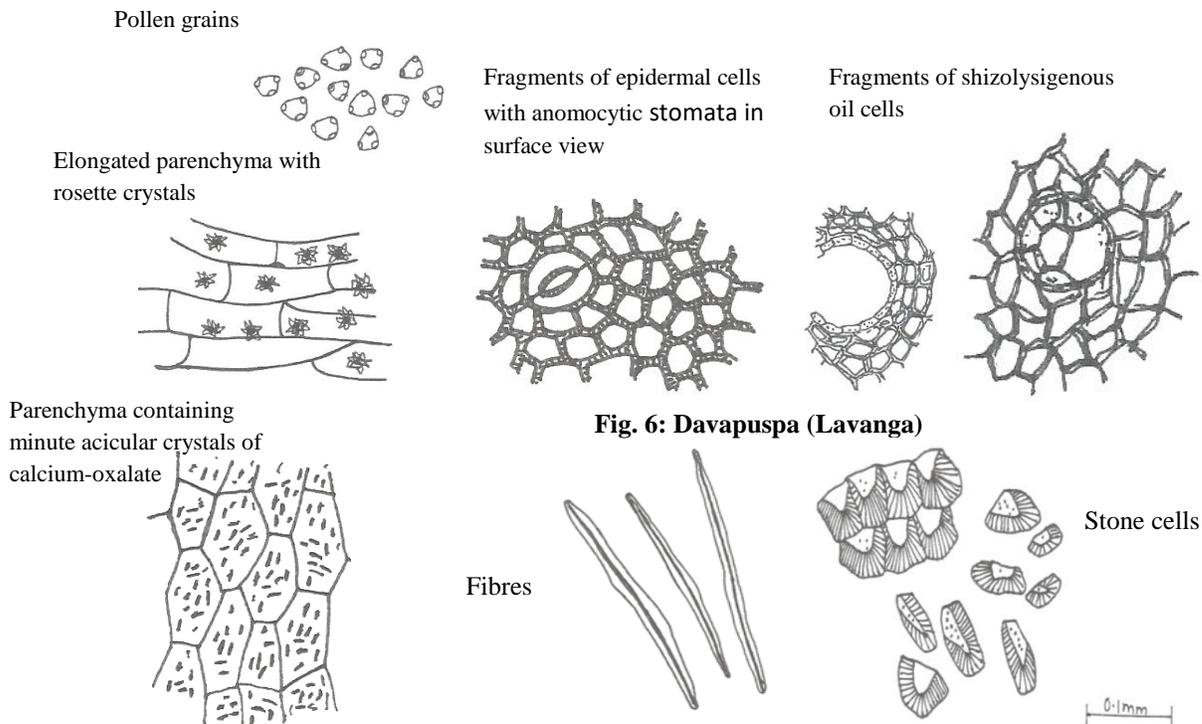


Fig. 6: Davapuspa (Lavanga)

Fig.7: Tvaka

Plate 3: TLC finger prints

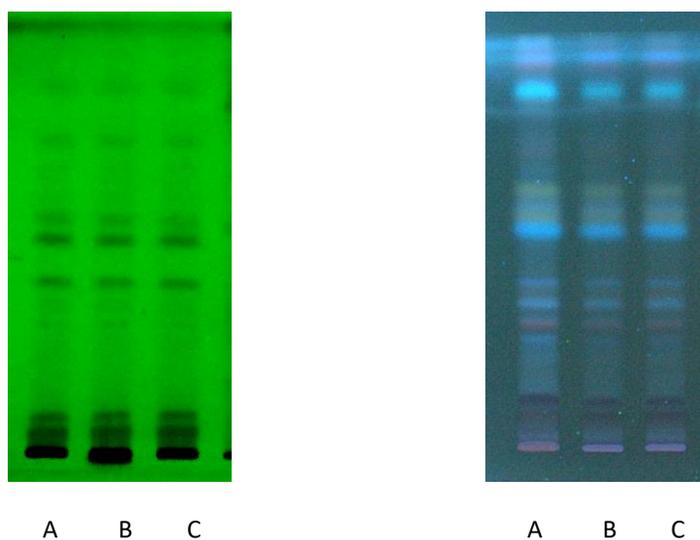


Fig. 1-2: TLC profile under 254 nm & 366nm Track 1: Batch A, Track 2: Batch B, Track 3: Batch C

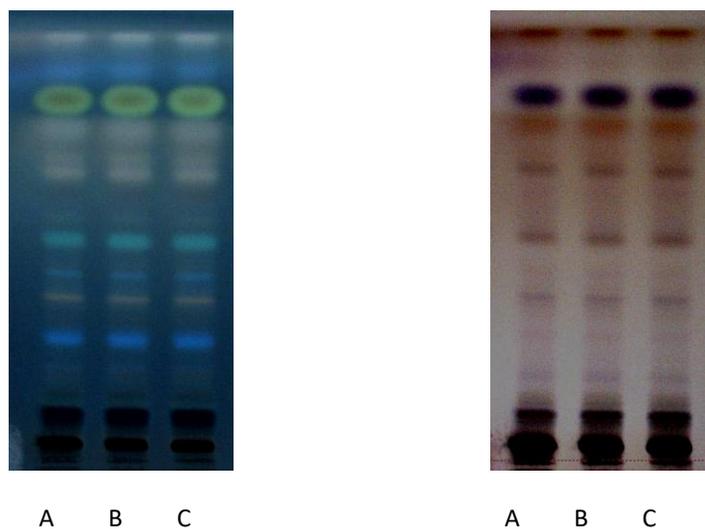


Fig. 3-4: *Dragon-dorff's* reagent under 366 nm & visible light Track 1: Batch A, Track 2: Batch B, Track 3: Batch C

Plate 4: Determination of microbial load

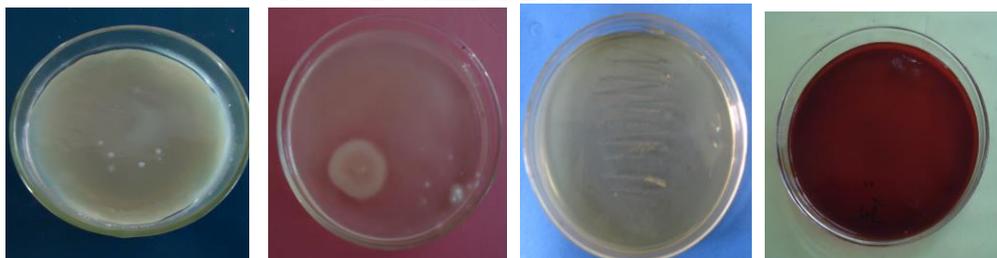


Fig. 1.1: Showing Total Bacteria count

Fig. 1.2: Showing Yeast & Moulds

Fig. 1.3: Showing negative result for *Pseudomonas aeruginosa*

Fig. 1.4: Showing negative Result for *Salmonella*



Fig. 1.5: Showing negative result for *E. coli*

Fig. 1.6: Showing negative result for *Staphylococcus aureus*

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