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Qualitative And Quantitative Analysis Of Several Herbal Extracts Acting As Anti-Acne Agents

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ABSTRACT-

Strong medicinal substances are developed in large part by the use of medicinal plants. Approximately 80% of people in underdeveloped nations still receive their main medical treatment from traditional medicine. Phytochemical screening of neem, licorice, cassia tora and hibiscus extracts reveals different profiles of bioactive compounds. Phenolics are present in neem and hibiscus, but not in licorice and Cassia Tora. Tannins are commonly found while steroids are found only in Neem and Cassia Tora. Alkaloids are found in all but licorice, and glycosides are found in neem and licorice, but not in Cassia Tora and Hibiscus. Flavonoids are the same in all extracts, suggesting a common phytochemical class. The evaluation parameters show that all four substances are solid, odorless and have a similar brown color. Their loss on drying ranges from 0.11 to 0.23% and the pH of their 1% aqueous solutions ranges from 3.2 to 4.3. Licorice has the highest yield (50%) and Cassia Toral the lowest (28%). Total ash content is highest in neem (8.5%) and acid insoluble ash content is highest in licorice (4%). Water-soluble ash varies slightly, with values for water-soluble extracts ranging from 7.8% for licorice to 9.5% for neem. Licorice contains the most alcohol-soluble extracts (3.8%). Solubility tests show that all substances are soluble in distilled water, ethanol, benzene and petroleum ether. In acetone, neem dissolves slightly, while others dissolve completely. Unlike others, licorice dissolves easily in chloroform. Toluene dissolves Neem, Cassia Tora, and Hibiscus but not Licorice, highlighting selective solubility and unique chemical interactions with solvents.

KEY WORDS-

Medicinal Plants, Extraction, HPLC Analysis, Solubility of Extract.

INTRODUCTION-

Medicinal plants play an important role in the development of potent therapeutic agents. Today estimate that about 80 % of people in developing countries still relays on traditional medicine based largely on species of plants and animals for their primary health care. Herbal medicines are currently in demand and their popularity is increasing day by day. About 500 plants with medicinal use are mentioned in ancient literature and around 800 plants have been used in indigenous systems of medicine [01].

Herbal drugs referred as plants materials or herbals, involves the use of whole plants or parts of plants, to treat injuries or illnesses. Herbal drugs are use of therapeutic herbs to prevent and treat diseases and ailments or to support health and healing. Herbal medicine is the study of pharmacognosy and the use of medicinal plants, which are the basis of traditional medicine. The major use of herbal medicine is for health promotion [02].

World Health Organization (WHO) has defined herbal are finished and labeled medicinal product that contain active ingredients, aerial and underground parts of the plants or other plant material or combination. Herbal medicine have a long history of use and better patient tolerance and public acceptance [03].

Medical plants have a renewable source, so that we can have sustainable supplies of cheaper medicine for the world's growing population. Because of the rich agro-climatic, culture and ethnic biodiversity of developing countries like India availability of medicinal plants is not a problem. The cultivation and processing of medicinal herbs are eco-friendly. Prolong and apparently uneventful use of herbal medicines is safe and efficacious.

Acne is the most common skin disease worldwide. It is estimated that 80-95% of all adolescents will have acne at some point in their lives, and in some cases, the acne will continue into adulthood. Genetics plays a role in the development of acne and males & females both are equally affected, but males tend to have more severe cases. Teenagers develop acne at a higher rate than any other age group. This is because hormone production during puberty increases the output of the sebaceous glands and the rate of skin-cell turnover within the follicles [04].

A number of factors contribute to the development of acne lesions, includes internal hormones, bacteria, some medications, certain chemicals/ products that come in contact with the skin, local pressure to the skin surface, and stress. While acne cannot be cured, it can be controlled. The goal of treating acne is to reduce the symptoms and to prevent permanent scarring. Acne arises from the interaction of the following factors includes increased sebum production caused by androgenic stimulation of sebaceous glands in puberty or adulthood, outlet obstruction of the sebaceous follicle arising from an abnormal keratinization process characterized by increased cohesiveness and turnover of follicular epithelial cells, \proliferation of P. acne, an anaerobic diphtheria residing in the sebaceous follicle [05].

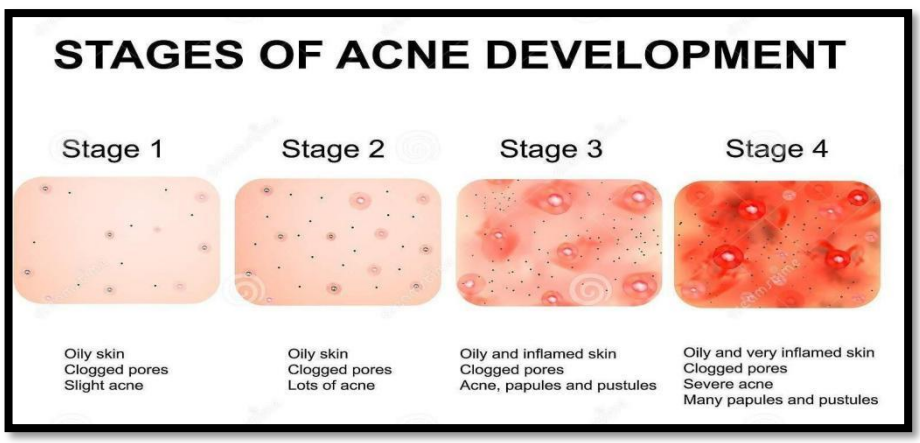


Fig No 01 – Stages of Acne Development

MATERIAL & METHODS-

The provided list includes a variety of drugs and chemicals along with their respective manufacturers or suppliers. The majority of these substances are supplied by LOBA Chemie Pvt Ltd, Palghar, which includes common laboratory chemicals such as Propylene Glycol, Triethanolamine, Methyl Paraben, Propyl Paraben, Dichloromethane, Methanol, Ether, Ethanol, Hydrochloric Acid, Sulphuric Acid, Ferric Chloride solution, and Fehling's Solution. On the other hand, Research Lab Pvt Ltd, located in Islampur, Sangali, supplies specific chemicals including Carbopol, Phenyl Hydrazine, Sodium Hydroxide, Dextrose, Agar, Ethyl Acetate, Chloroform, Alpha-naphthol, and Sodium Potassium Tartrate. These chemicals are essential for various pharmaceutical and laboratory applications, indicating a diverse range of chemical products supplied by these two companies.

PLANTS MATERIAL

Collection and drying of plant parts-

The fresh leaves of Neem leaves and Hibiscuse Flower were collected from local area of Nagpur district in the month of Jun 2020. While the Licorice root and Cassia Tora seed were purchased from the local market of Nagpur district. The fresh leaves of neem, licorice root, cassia tora seed, Hibiscuse flower were dried under shade and were powdered. The plant specimens were dried and there herbarium sheets were prepared as shown in result.

AUTHENTICATION OF HERBAL DRUGS-

Authenticated raw material is the basic starting point in developing a botanical product. In addition, each step of harvest, storage, processing and formulation may dramatically alter the quality and consistency of final product. Therefore methods to ensure quality control in manufacturing and storage are requisite tools to ensure optimal efficacy and safety of these products. Furthermore, such controls are critical for the evaluation of pharmacological, toxicological or clinical studies involving botanical products. Authentication is especially useful in cases of drugs that are frequently substituted or adulterated with other varieties which are morphologically and chemically indistinguishable. Several herbal drugs in the market still cannot be identified or authenticated based on their morphological or histological characteristics. Use of wrong drugs may be ineffective or it may worsen the condition.

PREPARATION OF EXTRACT [04-07]-

The fresh part of plant was dried in laboratory air dryer at 40°C for 7 days and pulverized using cutting mill. It was then stored in a desiccators at 2°C until further use. All solvents were double distilled before use. Dry powdered of plant was successively extracted with ethanol using Soxhlet apparatus for 6 hrs separately. The extracts were filtered and concentrated using a rotary evaporator. Final concentrated extracts upon freeze drying and ethanoilc extract were stored at 2°C in labeled sterile bottles and kept as aliquots until further evaluation. The extracts thus obtained were filtered, concentrated on water bath to thick paste and dried under vacuum.

EXTRACTION BY SOXHLET APPARATUS [08-10]-

Extraction may be defined as a process of removing the soluble active principles present in plant tissue from the inactive and insoluble residue by use of selective solvents. For continuous hot extraction on small scale, a Soxhlet apparatus is used. It consists of a flask, a Soxhlet extractor and a reflux condenser. The coarse powder of plant material was placed in a thimble made of filter paper was inserted in to the wide tube of the extractor. The solvent ether was taken in the flask to remove waxy substances from the plant.

The solvent was heated and the vapour arising from the flask got in to the condenser through the side tube and the liquid condensed from the vapours dripped into the thimble. The solvent liquid level slowly raised and during this period, fat got extracted of its soluble constituents. When the level of the liquid reached the top of the siphon, it got siphoned in to the flask. Again a portion of solvent from the solution was vaporized leaving the constituents in the flask itself and the process mentioned above was repeated. The plant was removed from the thimble and dried in open atmosphere. Petroleum ether from the plant was removed after the plant was ready to treat with ethanol to extract the constituents from the plant. The solvent ethanol was taken in the flask and it was heated. The same process was repeated again and again until all the solutes were extracted. The active constituents containing extractive liquid was processed with rotary film evaporator to remove the solvent ethanol and the pure extract (i.e. 100% free from solvents) was collected from the flask.

The extract may contain different active constituents; it was identified with the help of Infrared studies, chemical tests, and solubility test and absorption maxima of the extract.

Pharmacognostic studies [11]-

Pharmacognostic evaluation helps to screen the commercial varieties, substitutes, adulterants and any other quality control of the drugs. It is a simple and reliable tool, helps to obtain information about biochemical and physical properties of crude drug. The application of pharmacognostic protocols such as macro morphology, microscopy, organoleptic tests, ash value study will help in identifying genuine drugs because these tests result in specific results for a particular drug.

Solubility Test [12]-

5gm of fine powder of extract was added to the different solvent taken in a test tube and mixed well and allowed to stand period. Then the mixture was filtered through filter paper kept in different funnels. The filter paper that contain fewer residues considered as more soluble in that solvent.

Preliminary phytochemical evaluation [13-20]-

Preliminary screening of phytochemical is a valuable step, in the detection of the bioactive principles present in medicinal plants and subsequently may lead to drug discovery and development. The chemical test for phytochemical in extracts of leaves of Neem leaves, Licoricer root, Cassia tora seed, Hibisause flower were carried out as described below and result were recorded.

TEST FOR CARBOHYDRATES:-

Fehling's test-

Mix 1ml. Fehling's A and 1ml. Fehling's B solutions boil for one minute. Add equal volume of test extract solution. Heat in boiling water bath for 5-10 min. Appearance of orange red precipitate indicates presence of carbohydrates.

Benedict's test-

Mix equal volume of Benedict's reagent and test extract in test tube. Heat in boiling water bath for 5 min. Solution appears green, yellow or red depending on amount of reducing sugar present in test solution.

TEST FOR PROTEINS-

Biuret test-

Add 2ml of Biuret reagent to 2ml of extract. Shake well and warm it on water bath. Appearance of red or violet colour indicates presence of proteins. To 3 ml. extract add 4% NaOH and few drops of 1% CuSO4 solution. Violate or pink colour appears.

Million's test-

Test solution treated with million's reagent and heated on a water bath, a white ppt is formed.

Xanthoprotein test-

Test solution treated with conc. nitric acid and on boiling gives yellow precipitate.

Ninhydrine test-

Test solution treated with Ninhydrine reagent gives blue colour.

TEST FOR AMINO ACIDS:-

Ninhydrine test-Heat 3 ml extract and 3 drops of 5% Ninhydrine solution in boiling water bath 10 min. Purple or bluish color appears.

Test for Tyrosine- Heat 3 ml extract and 3 drops of Million's reagent. Solution shows dark red color.

Test for cysteine- To 5 ml of extract add few drops of 40% NaOH and 10% lead acetate solution. Boil. Black ppt. of lead sulfate is formed.

TEST FOR STEROIDS-

Salkowski reaction- To 2 ml of extract, add 2 ml chloroform and 2 ml of conc. H2SO4. Shake well. Chloroform layer appears red and acid layer shows greenish yellow fluorescence.

Liebermann's reaction- Mix 3 ml extract with 3 ml acetic anhydride. Heat and cool. Add few drops of conc. H2SO4. Blue color appears.

TEST FOR SAPONINS-

Foam test in that a small amount of extract taken in a test tube with little quantity of water. Shake vigorously. Appearance of foam persisting for 10 minutes indicates presence of Saponin.

TEST FOR ALKALOIDS-

Dragendroff's test: - Dissolve extract of the herbal drug in chloroform. Evaporate chloroform and acidify the residue by adding few drops of Dragendroff's reagent (Potassium Bismuth Iodide). Appearance of orange red precipitate indicates presence of alkaloids.

Mayer's test:-2-3 ml of filtrate with few drops of Mayer's reagent gives ppt. 3. Wagner's test: - 2- 3 ml of filtrate with few drops of Wagner's reagent gives reddish brown colour.

Murexide test for purine alkaloid: -To 3-4 ml. test solution add 3-4 drops of conc.HNO3. Evaporate to dryness. Cool and add 2 drops of NH4OH. Purple colour is observed.

TEST FOR FLAVANOIDS-

Ferric chloride test: -To the alcoholic solution of the extract add few drops of neutral ferric chloride solution. Appearance of green colour indicates presence of flavanoids.

Shinoda Test:- To dry extract, add 5 ml. of 95% ethanol, few drops conc. HCL and 0.5 g magnesium turnings. Pink colour observed.

Zinc-hydrochloric acid-reduction test: -Test solution with zinc dust and few drops of HCL shows magneta red colour

Alkaline reagent test: -Test solution when treated with sodium hydroxide solution shows increase in the intensity of yellow colour which becomes colourless on addition of few drops of dilute acid.

Lead acetate solution test: -Test solution with few drops of lead acetate solution (10%) gives yellow precipitate.

TEST FOR GLYCOSIDES-

Baljets test: - Treat the extract with sodium picrate solution. Appearance of yellow to Orange colour indicates presence of glycoside with lactone ring.

Keller-killiani test:- The test solution with few drops of glacial acetic acid in 2 ml of ferric chloride solution and conc. sulphuric acid is added from the sides of test tube which shows the separation between two layers, lower layer shows reddish brown and upper layer turns bluish green.

Bromine water test:-Test solution dissolved in Bromine water gives yellow precipitate. **Legal's test: -** Test solution when treated with pyridine (made alkaline by adding sodium nitroprusside solution) gives pink to red colour.Ash determination

After ignition the remaining material is known as ash. There are two types of ash one is the ash derived from plant tissues (physiological ash) and the other one is residue of extraneous matter adhering to plant surface (non-physiological ash).Ash is determined by following three methods.

Total ash [20-22]

Weight of residue obtained after ignition. About 2gm of powdered drug was weighed accurately into a tarred silica crucible. Incinerated at 450°C in a muffle furnace until free from carbon. The crucible was cooled and weighed. Percentage of total ash was calculated with reference to air-dried substance. Determination of total ash value formula. Total ash value of the sample = 100 (z-x) % / y. X= weight of empty dish. Y= weight of the drug taken. Z= weight of the dish + ash (after complete incineration).

Acid insoluble ash [22-23]

Weight of residue obtained after boiling the total ash with dilute hydrochloric acid and igniting the remaining insoluble matter. The method of analysis is as follows. Ash obtained from the total ash was boiled with 25ml of 2N HCl for a few minutes. Filtered through an ash less filter paper. The filter paper was transferred into a tarred silica crucible.

Water soluble ash [21-23]

Difference in the weight between the total ash and the residue after treatment of total ash with water. The method is as follows Ash obtained from the total ash was boiled with 25 ml of distilled water for a few minutes and filtered through an ash less filter paper. The filter paper was transferred into a tarred silica crucible. Incinerated at 450°C in a muffle furnace until free from carbon. The crucible was cooled and weighed. Percentage of water-soluble ash was calculated with reference to air-dried substance.

Extractive value [24]

Extractive value determines the amount of active constituents extracted with solvent from a given amount of medicinal plant. It gives an idea about the nature of the chemical constituents present.

Determination of alcohol soluble extractive value [22-25]

About 5gms of air dried coarse powdered drug was weighed and macerated with 100ml of 90% alcohol in a closed flask for 24 hours, shaking frequently during the first 6 hrs and these allowed standing for 18 hrs. Thereafter it was filtered rapidly taking precautions against loss of the solvent. 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°C and weighed. The %age of the alcohol soluble extractive values was calculated with reference to the air-dried drug.

Determination of water soluble extractive value [26]-

About 5gm of air-dried powdered drug was taken & macerated with 100 ml of chloroform water in a closed flask for 24 hrs shaking frequently during the first 6 hrs and then allowed to stand for 18 hrs. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°C and weighed. The percentage of the water soluble extractive value was calculated with reference to the air-dried drug.

Ultraviolet-visible absorption spectroscopy [27]-

Determination of λ-max (absorption maxima)-

The stock solution was further diluted this solation was scanned at wavelength of 200-600 nm against blank. The wavelength of maximum absorbance was found.

Preparation of calibration curve-

Accurately weighted 10mg quantity of the drug was added in 100ml of methanol. These stock solution 100ug/mal were diluted with respective solvent give the series of solution containing the concentration of drug in the range of 10, 20, 30, 40, 50 ug/ml. The individual solvent were used as blank. Graph was plotted for absorbance Vs Concentration.

Identification (HPTLC) [20-27]-

High-performance thin-layer chromatography (HPTLC) is an enhanced form of thin-layer chromatography (TLC). A number of enhancements can be made to the basic method of thin-layer chromatography to automate the different steps, to increase the resolution achieved, and to allow more accurate quantitative measurements. Automation is useful to overcome the uncertainty in droplet size and position when the sample is applied to the TLC plate by hand. One approach to automation has been the useof piezoelectric devices and inkjet printers for applying the sample.

The spot capacity (analogous to peak capacity in HPLC) can be increased by developing the plate with two different solvents, using two-dimensional chromatography. The procedure begins with development of sample loaded plate with first solvent. After removing it, the plate is rotated 90° and developed with a second solvent.

A) Neem-

Procedure for the neem Neem:

Preparation of Sample Solution:

Weigh accurately about 0.25 mg extract mix with 20ml of methanol and transfer into a 10 ml volumetric flask. Dilute up to mark with methanol. Filter the solution through whatman filter paper. Sonicate for 15 minutes and then apply. Use the resulting solution as sample solution.

Preparation of Reference Sample Solution-

Weigh accurately about 1g of herb powder of reference sample and transfer into a 25 ml volumetric flask; add about 25 ml of Methanol in it. Sonicate for 15 minutes. Dilute up to the mark with Methanol. Filter the solution through Whatman filter paper. Use the resulting solution as reference sample solution.

Preparation of Black Sample Solution:

Add about 20 ml of Methanol in it. Sonicate for 15 minutes. Dilute up to the mark with Methanol. Filter the solution through Whatman filter paper. Use the resulting solution as blank sample solution.

Table no 01 - Parameters for the HPLC analysis

Sr no	Parameters	Specification
1.	Stationary Phase	Silica Gel 60 F254 TLC Plate
2.	Mobile phase	Toluene: Ethyl acetate: n- butanol: Formic acid: Water (2.5, 1.5, 0.5, 0.5)
3.	Sample application	Apply 10 µl of reference sample and test solution on TLC plate.
4.	Developing distance	10 cm
5.	Saturation Time	20 min.
6.	Spraying reagent	Anisaldehyde H_2SO_4

B) Licorice Root-

Preparation of Test Solution:

Weigh accurately about 0.25 mg extract mix with 20ml of methanol and transfer into a 10 ml volumetric flask. Dilute up to mark with methnol. Filter the solution through whatman filter paper. Sonicate for 15 minutes and then apply. Use the resulting solution as sample solution.

Preparation of Reference Sample Solution-

Weigh accurately about 1g of herb powder of reference sample and transfer into a 25 ml volumetric flask; add about 25 ml of Methanol in it. Sonicate for 15 minutes. Dilute up to the mark with Methanol. Filter the solution through Whatman filter paper. Use the resulting solution as reference sample solution.

Preparation of Black Sample Solution-

Add about 20 ml of Methanol in it. Sonicate for 15 minutes. Dilute up to the mark with Methanol. Filter the solution through Whatman filter paper. Use the resulting solution as blank sample solution.

Table no 02 - Parameters for the HPLC analysis

Sr no	Parameters	Specification
1.	Stationary Phase	Silica Gel 60 F254 TLC Plate
2.	Mobile phase	n- butanol: water: Acetic acide(7:2:1)
3.	Sample application	Apply 10 µl of reference sample and test solution on TLC plate.
4.	Developing distance	10 cm
5.	Saturation Time	20 min.
6.	Spraying reagent	Anisaldehyde H2SO4

C) Cassia Tora Seed-

Preparation of Sample Solution-

Weigh accurately about 0.25 mg extract mix with 20ml of methanol and transfer into a 10 ml volumetric flask. Dilute up to mark with methanol. Filter the solution through whatman filter paper. Sonicate for 15 minutes and then apply. Use the resulting solution as sample solution.

Preparation of Reference Sample Solution-

Weigh accurately about 1g of herb powder of reference sample and transfer into a 25 ml volumetric flask; add about 25 ml of Methanol in it. Sonicate for 15 minutes. Dilute up to the mark with Methanol. Filter the solution through Whatman filter paper. Use the resulting solution as reference sample solution.

Preparation of Black Sample Solution-

Add about 20 ml of Methanol in it. Sonicate for 15 minutes. Dilute up to the mark with Methanol. Filter the solution through Whatman filter paper. Use the resulting solution as blank sample solution.

Table no 03 - Parameters for the HPLC analysis

Sr no	Parameters	Specification
1.	Stationary Phase	Silica Gel 60 F254 TLC Plate
2.	Mobile phase	Toluene: Ethyl acetate (9:1)
3.	Sample application	Apply 10 µl of reference sample and test solution on TLC plate.
4.	Developing distance	10 cm
5.	Saturation Time	20 min.
6.	Spraying reagent	Anisaldehyde H2SO4

RESULT & DISCUSSION –

Solubility-

Table No 04- Solubility of extract in various solvent

Solvent	Neem	Liquorice	Cassia Tora	Hibiscuse
Distilled Water	Soluble	Soluble	Soluble	Soluble
Ethanol	Soluble	Soluble	Soluble	Soluble
Acetone	Slightly	Soluble	Soluble	Soluble
Chloroform	Soluble	slightly	Soluble	Soluble
Benzene	Soluble	Soluble	Soluble	Soluble
Toluene	Soluble	Insoluble	Soluble	Soluble
Pet. ether	Soluble	Soluble	Soluble	Soluble

The solubility of Neem, Liquorice, Cassia Tora, and Hibiscus in various solvents shows distinct patterns. All four substances are soluble in distilled water, ethanol, benzene, and petroleum ether, indicating a general compatibility with both polar (distilled water and ethanol) and non-polar (benzene and petroleum ether) solvents. In acetone, only Neem is slightly soluble, while the others are fully soluble, suggesting Neem's partial compatibility with this solvent. In chloroform, Liquorice is only slightly soluble, differing from the other substances which are soluble, indicating a unique interaction. Toluene dissolves Neem, Cassia Tora, and Hibiscus, but not Liquorice, highlighting its selective solubility. These differences reflect the unique chemical properties and interactions of each substance with the solvents.

Standizations of Extracts-

Table no 05 –The Standizations Evaluation Parameter of various Extracts

Evaluation Parameter	Neem	Liquorice	Cassia Tora Seed	Hibiscuse flower
Nature	Solid	Solid	Solid	Solid
Colour	Brown	Light Brown	Brown	Brown
Taste/odour	Odourless	Odourless	Odourless	Odourless
Loss on drying(at 105oC)	0.15%	0.11%	0.23 % w/w	0.12 %
pH of 1%w/v aq. solution	3.4	4.2	3.2	4.3
Estimation of % yield	40%	50 % w/w	28 % w/w	30 % w/w
Total Ash	8.5 % w/w	6.5 % w/w	6.12 % w/w	6.12 % w/w
Acid insoluble ash	2.5 % w/w	4 % w/w	1.08 % w/w	1.08 % w/w
Water soluble ash	0.5 % w/w	1 % w/w	1.22 % w/w	1.22 % w/w
Water soluble extractive value	9.5 % w/w	7.8 % w/w	8.2 % w/w	8.2 % w/w
Alcohol soluble extractive value	2.5 % w/w	3.8 % w/w	3.1 % w/w	3.1 % w/w

Neem, Liquorice, Cassia Tora Seed, and Hibiscus flower share several evaluation parameters, all being solid and odourless with similar brown coloration. Their loss on drying at 105°C ranges from 0.11% to 0.23%, indicating slight moisture retention variability. The pH of their 1% aqueous solutions varies from 3.2 to 4.3, reflecting slight acidity. Yield percentages show Liquorice as the highest (50%) and Cassia Tora as the lowest (28%).

Total ash content, indicating mineral residue after combustion, is highest in Neem (8.5%) and equal in Cassia Tora and Hibiscus (6.12%). Acid-insoluble ash, representing silicate impurities, is highest in Liquorice (4%) and lowest in Cassia Tora and Hibiscus (1.08%). Water-soluble ash, indicative of soluble minerals, varies slightly among the samples, with Neem having the least (0.5%). Water-soluble extractive values, reflecting the amount of water-extractable components, range from 7.8% in Liquorice to 9.5% in Neem.

Alcohol-soluble extractive values, which measure components extractable by alcohol, are highest in Liquorice (3.8%) and similar in Cassia Tora and Hibiscus (3.1%). These variations underscore the unique chemical profiles and extractive efficiencies of each substance.

Phytochemical screening of extract

The following phytoconstituents were found to present in the ethanolic extract of neem, liquorice, cassia tora, hibiscuse respectively.

Table No 06- Phytochemical screening of extract

Test	Neem	Liquorice	Cassia Tora	Hibiscuse
Phenols	+	-	-	+
Tannins	+	+	+	+
Steroids	+	-	+	-
Alkaloids	+	-	+	+
Glycosides	+	+	-	-
Flavonoids	+	+	+	+

The phytochemical screening of Neem, Liquorice, Cassia Tora, and Hibiscus extracts reveals distinct profiles for various bioactive compounds. Phenols are present in Neem and Hibiscus but absent in Liquorice and Cassia Tora. Tannins are universally present across all four extracts. Steroids are found in Neem and Cassia Tora, but not in Liquorice and Hibiscus. Alkaloids are detected in Neem, Cassia Tora, and Hibiscus, while Liquorice lacks these compounds. Glycosides are present in Neem and Liquorice but are absent in Cassia Tora and Hibiscus. Flavonoids are consistently present in all four extracts, indicating a commonality in this class of phytochemicals. This screening highlights the unique and shared chemical constituents among these plant extracts, reflecting their potential diverse therapeutic properties.

Standard calibration cure of extract-

Table no 07– The observations of Standard calibration cure of extract

Concentration (ug/ml)	Absorbance		
	Neem	Liquorice	Cassia Tora
0	0	0	0
10	0.185	0.063	0.09
20	0.366	0.094	0.189
30	0.545	0.161	0.271
40	0.7	0.208	0.369
50	0.9	0.253	0.453

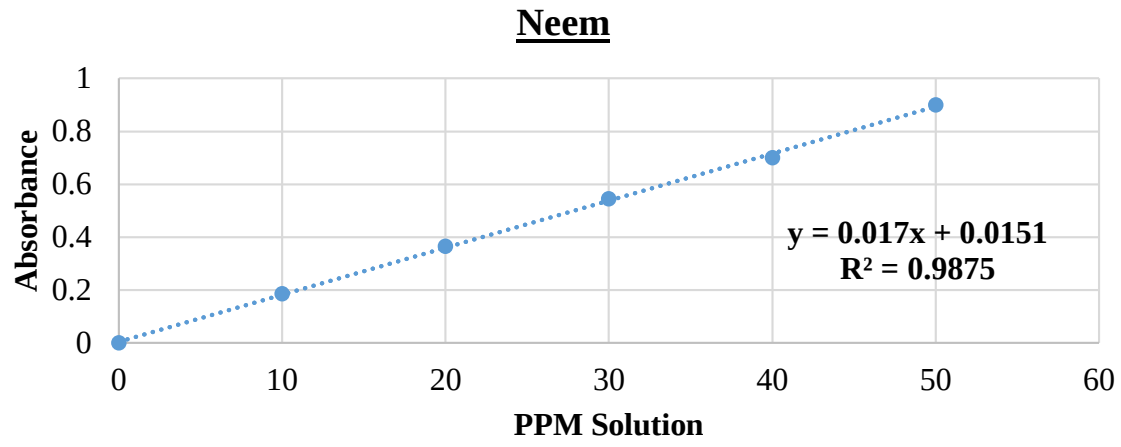


Fig no 02- Calibration Curve Neem Extract

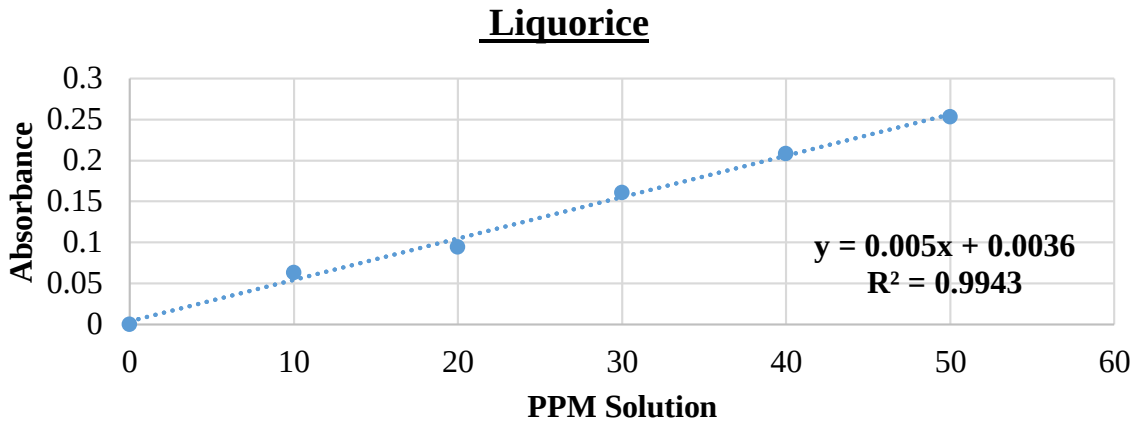


Fig no 03- Calibration Curve Liquorice Extract

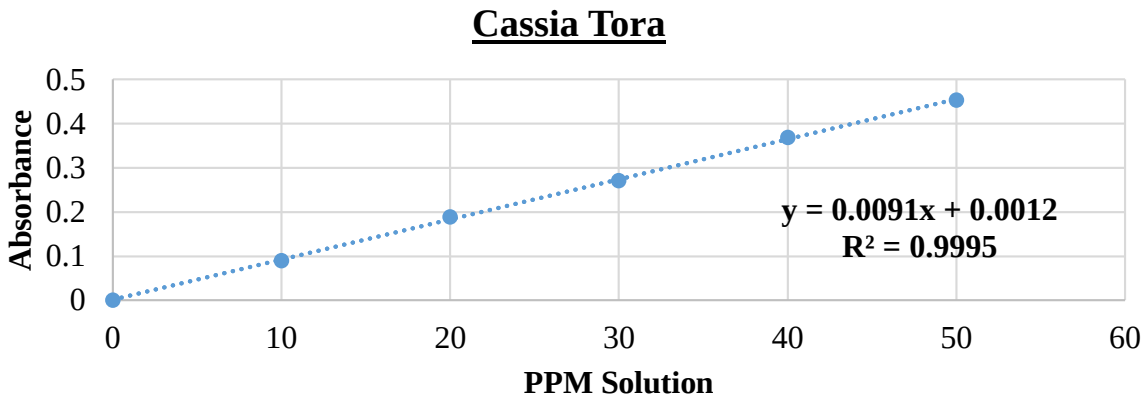
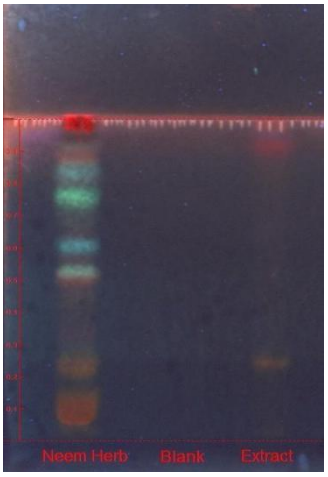
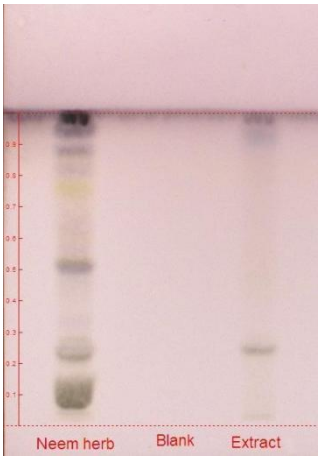


Fig no 04- Calibration Curve Cassia Tora Extract

HPLC Analysis-

1. **Neem Extract-**





After Derivatization At 366 Nm	After Derivatization In White R

Fig no 04- Neem leaves extract visualization

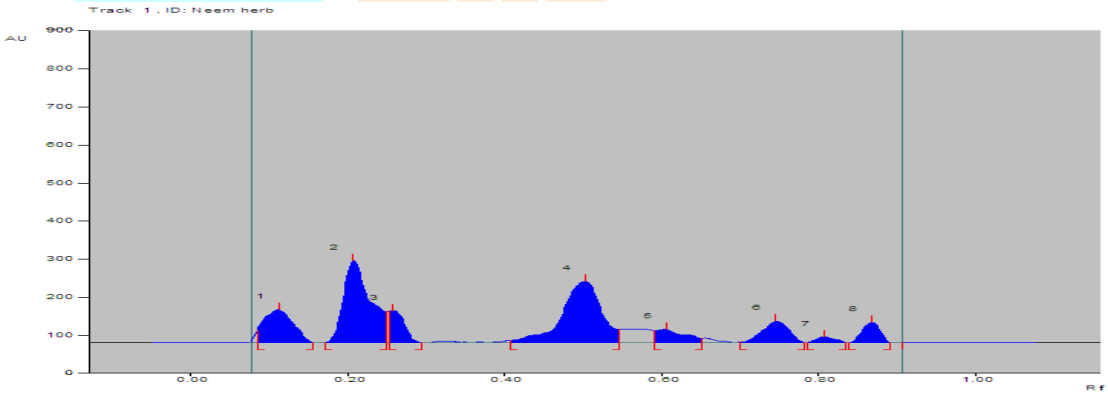


Fig no 05 - Chromatogram of Inhouse Reference Neem leaves sample solution

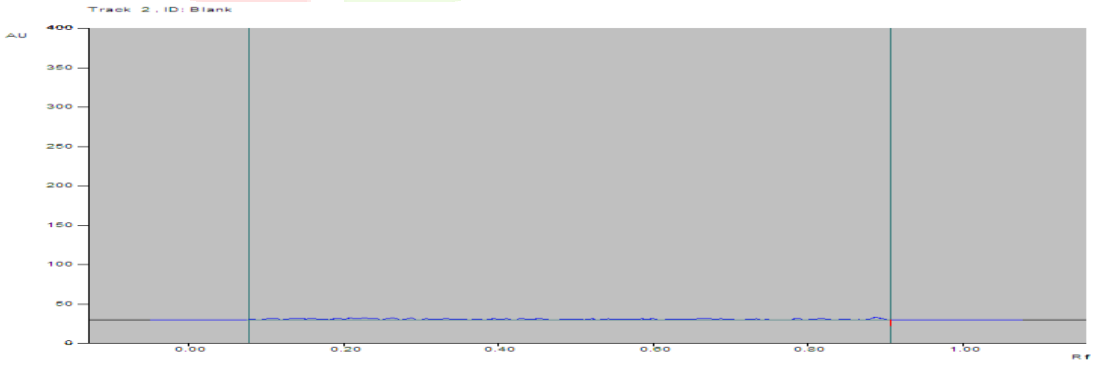


Fig no 06 -Chromatogram of blank sample solution

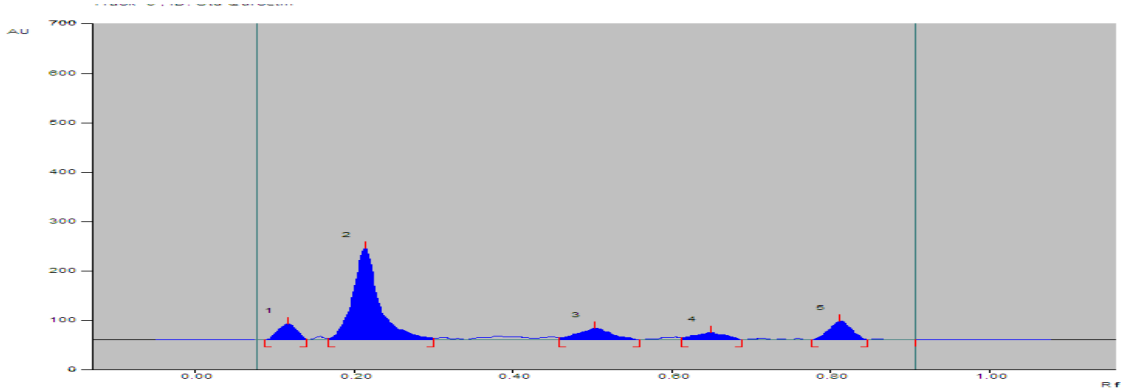
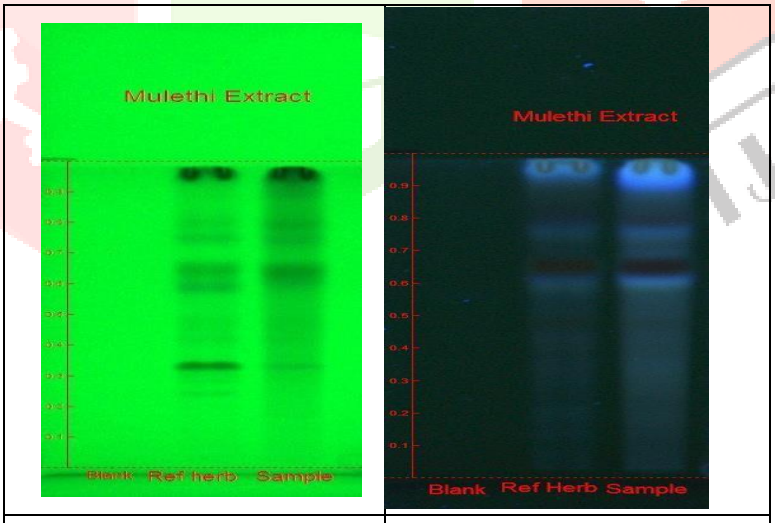


Fig no 07 -Chromatogram of Neem leaves extract solution

At 366 nm, the chromatogram of the test solutions displays the band similar in position and colour to that seen in standard solution and reference sample solution with respect to principal bands. The chromatograms illustrate the phytochemical profiles of three samples: Cassia Tora Herb, Neem Herb, and a blank. The first chromatogram of Cassia Tora Herb shows five peaks, with the second peak being the most prominent, indicating the major constituent. The second chromatogram of Neem Herb displays eight peaks, with significant peaks at the second and fourth positions, highlighting its key compounds. The third chromatogram, representing the blank sample, shows no significant peaks, confirming the absence of detectable compounds or contaminants. These chromatograms provide a comparative analysis of the chemical compositions of the herbal extracts and verify the accuracy of the chromatographic method using the blank sample.

2. Liquorice-



At 254 nm	At 366 nm

Fig no 08- Liquorice root extract visualization

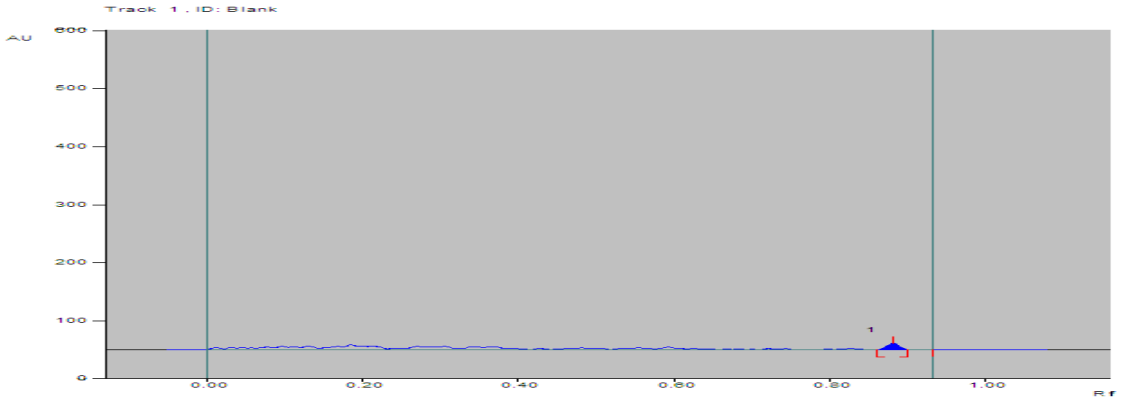


Fig no 09- Chromatogram of blank sample solution

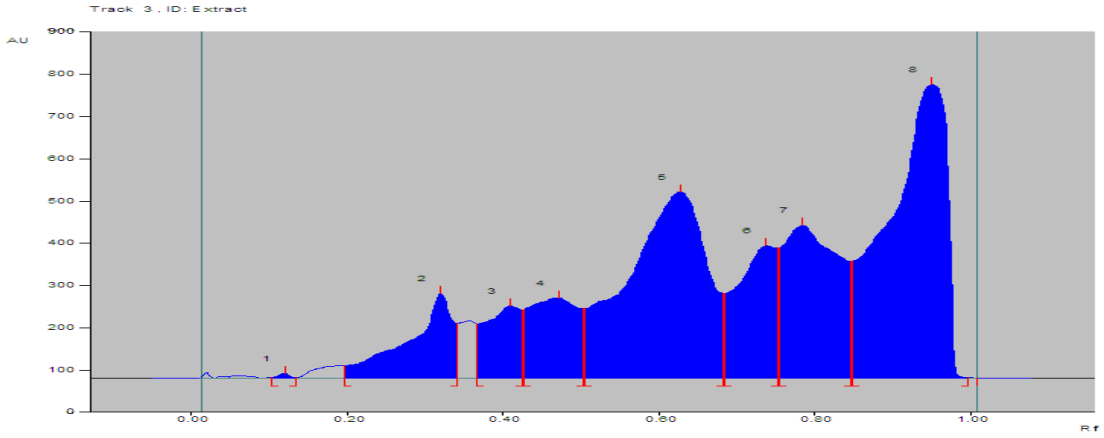


Fig no 10- Chromatogram of Mulethi (Liquorice root) extract sample solution

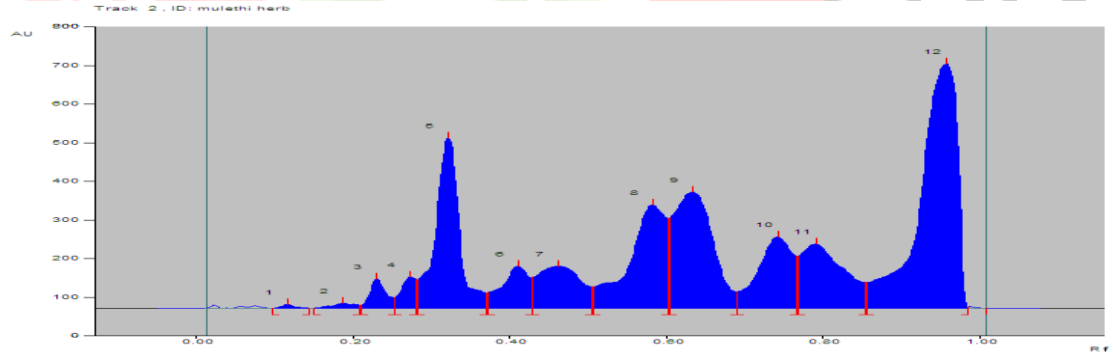
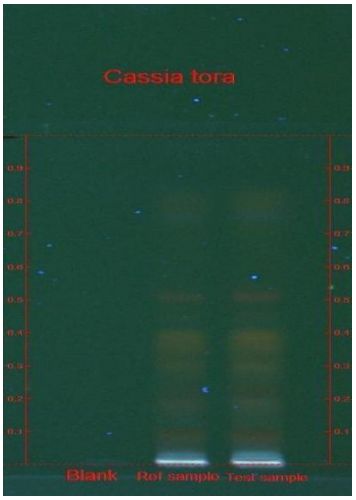
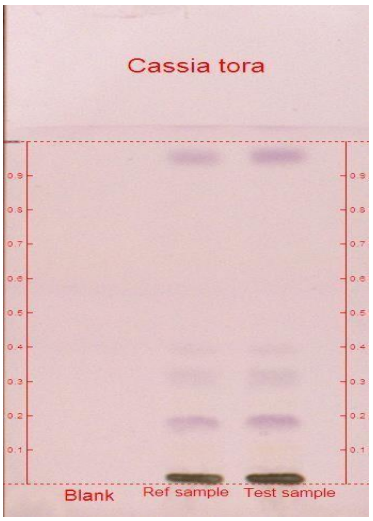


Fig no 11- Chromatogram of In-house Reference Mulethi (Liquorice root) sample solution

At 366 nm, the chromatogram of the test solutions displays the band similar in position and colour to that seen in standard solution and reference sample solution with respect to principal bands. There are distinct peaks, each representing different compounds within the various extract. The height of each peak correlates with the concentration of the respective compound, with higher peaks indicating higher concentrations, with the highest absorbance, signifies the most abundant compound in the extract. The variation in peak heights and retention times reveals the complex phytochemical composition of the various herb.

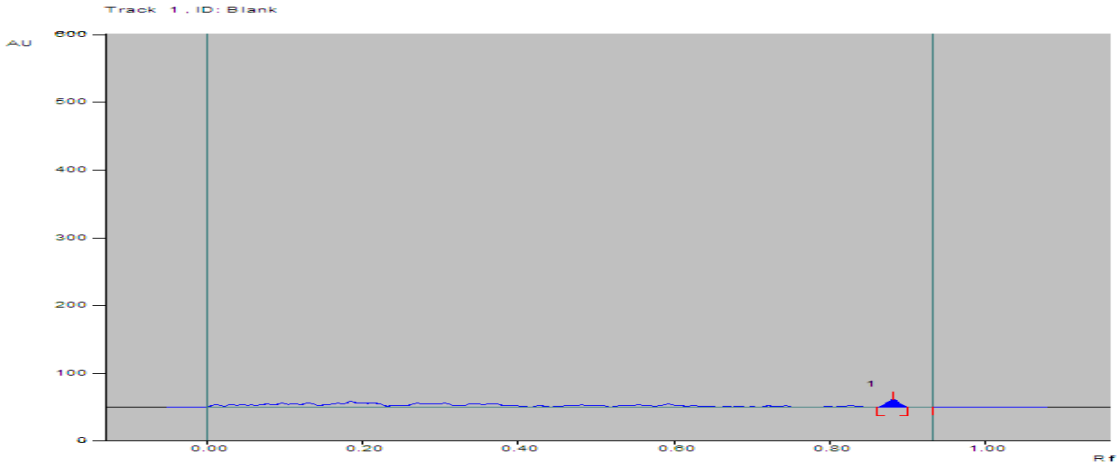
3. Cassia Tora Seed-





At 366 nm	After derivatization in White R

Fig no 12- Cassia tora extract



Fog no 13 -Chromatogram of blank sample solution

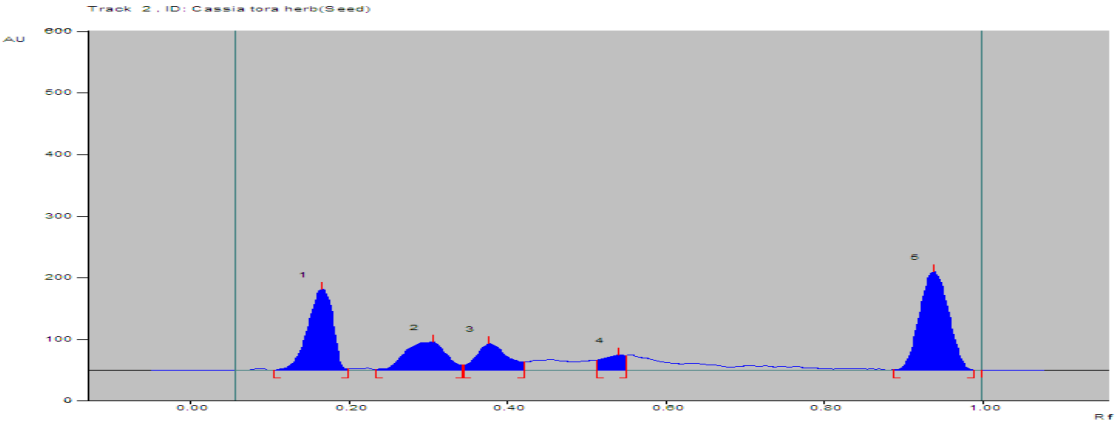


Fig no 14 - Chromatogram of Inhouse Reference Cassia tora (seed)sample solution

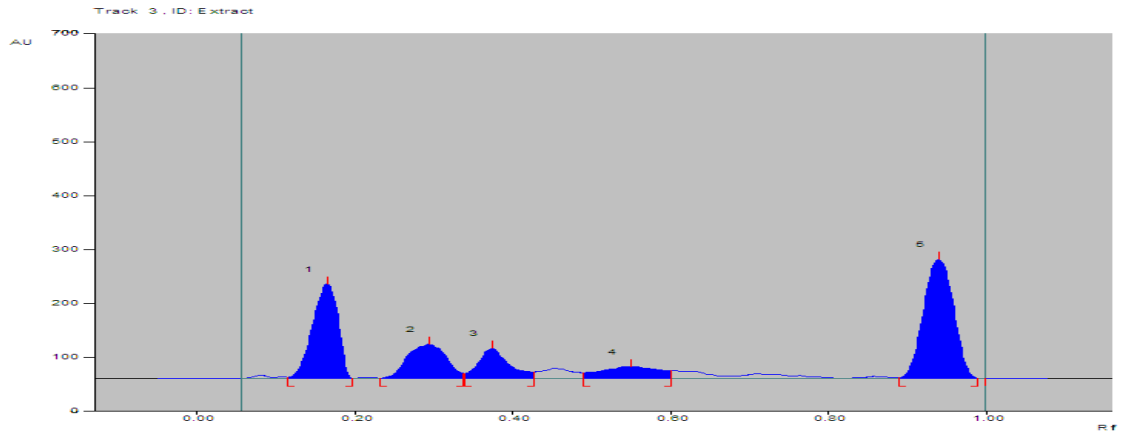


Fig no 15- Chromatogram of Cassia tora (seed) extract sample solution

At 366 nm, the chromatogram of the test solutions displays the band similar in position and colour to that seen in standard solution and reference sample solution with respect to principal bands.

CONCLUSION-

The chromatograms of Cassia Tora Herb, Neem Herb, and a blank sample at 366 nm reveal distinct phytochemical profiles. The Cassia Tora chromatogram shows five peaks, with the second being the most prominent, indicating the major constituent. The Neem chromatogram displays eight peaks, with significant peaks at the second and fourth positions, highlighting key compounds. The blank chromatogram shows no significant peaks, confirming the absence of detectable compounds or contaminants. These chromatograms, by matching the band positions and colors with standard and reference samples, confirm the identity and concentration of various compounds within the herbal extracts. Phytochemical screening of Neem, Liquorice, Cassia Tora, and Hibiscus extracts shows varied bioactive compound profiles. Phenols are found in Neem and Hibiscus but not in Liquorice and Cassia Tora. Tannins are universally present, while steroids are found only in Neem and Cassia Tora. Alkaloids are present in all except Liquorice, and glycosides are found in Neem and Liquorice but not in Cassia Tora and Hibiscus. Flavonoids are consistent across all extracts, indicating a commonality in this phytochemical class. Evaluation parameters indicate that all four substances are solid, odourless, and have similar brown coloration. Their loss on drying ranges from 0.11% to 0.23%, and the pH of their 1% aqueous solutions varies from 3.2 to 4.3. Liquorice shows the highest yield (50%), and Cassia Tora the lowest (28%). Total ash content is highest in Neem (8.5%), and acid-insoluble ash is highest in Liquorice (4%). Water-soluble ash varies slightly, and water-soluble extractive values range from 7.8% in Liquorice to 9.5% in Neem. Alcohol-soluble extractive values are highest in Liquorice (3.8%). Solubility tests reveal all substances are soluble in distilled water, ethanol, benzene, and petroleum ether. In acetone, Neem is slightly soluble, while others are fully soluble. In chloroform, Liquorice is slightly soluble, differing from the others. Toluene dissolves Neem, Cassia Tora, and Hibiscus, but not Liquorice, highlighting selective solubility and unique chemical interactions with solvents.

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