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Extraction from Different Natural Sources and their Pharmacognostical Studies

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Abstract

Natural products from medicinal plants, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. The focus of this paper is on the analytical methodologies, which include the extraction, isolation and characterization of active ingredients in botanicals and herbal preparations. The analysis of Phytoconstituents was performed physicochemical parameters (foreign matter, moisture content, loss on drying, total ash, water-soluble ash, acid-insoluble ash, sulphated-ash, alcohol-soluble extractive, water-soluble extractive and pH values), Preliminary Phytochemical Screening (Test for carbohydrates, Test for amino acids, Test for steroid, Test for fat, Test for oils, Test for alkaloids, Test for tannins, Test for phenolic compounds, Test for Saponin glycosides, Test for flavonoids, Test for volatile oils and Test for heavy metals), Tests for Aflatoxin and Thin-Layer chromatography. The results were discussed that all Phytoconstituents extracted from Fennel, Amla and Tulsi were nom-toxic and safe. All Phytoconstituents were free from heavy metals and Aflatoxin. The major goals of extraction are to increase yields of bioactive substances, reduce extraction time, be more environmentally friendly, and attain economic viability without sacrificing biological activity.

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Keywords: *Plant Extraction, Foeniculum vulgare, Emblica officinalis, Ocimum sanctum, Natural products*

1. Introduction

It is feasible to extract and treat medicinal plants for use as herbal or conventional medication or to prepare them for experimentation. The process of getting a medicinal plant ready for experimental usage comprises harvesting it in the right time and place, having it authenticated by a professional, drying it properly, and grinding it. The bioactive component is subsequently isolated, fractionated, and removed as necessary. Additionally, it entails determining the quantity and kind of bioactive compounds that are present [1,2,3,4,5]. The preparation of medicinal plants for experimentation is the first and most crucial stage in generating high-quality research findings. It involves extracting the bioactive ingredients and evaluating their quantity and quality before continuing with the intended biological testing. Two broad kinds of phytochemicals are produced during a plant's life [6]. The principal metabolites are included in the first group. These are necessary for a plant to grow and develop normally. They include the chemicals essential for all plants' growth and development, such as growth regulators and cell wall constituents, as well as nucleic acids, carbohydrates, fatty acids, proteins, and other compounds. The second category consists of the secondary metabolites needed by the plant to improve its capacity to endure in its niche and fend off threats. In other words, secondary metabolites are chemicals that plants create to help them adapt to their environment. It is important to note that secondary metabolites play a variety of roles in the physiology and biochemistry of plants. Any plant species that grows in an unfavorable environment, such tepid and aquatic tropical forests, will make an effort to survive by creating biomolecules that could be insecticides, fungicides, antibacterial agents, or antiviral agents [7]. However, if a plant's leaves don't show any signs of invasion, they might include bioactive chemicals that act as a defence against insects and microbes. Due to the abundance of harmful fungi in the soil, the roots may frequently produce antifungal phytochemicals. In opposition to human pathogenic fungus, these secondary metabolites may also elicit antifungal responses [7]. Therefore, pharmacologists and

biochemists have a special interest in plant phytochemicals because of the variety of roles that these compounds play in plant cells. It is sufficient to mention that particular secondary metabolites are bioactive substances that have toxicological or pharmacological effects on both humans and animals. Terpenes and terpenoids, alkaloids, and phenolic compounds can all be generically categorised as bioactive phytochemicals [8]. Their unique structural traits depend on the chemical pathway that led to their biosynthesis. The process of extraction is crucial to the study of natural products. Continuous work is being done to advance and find better extraction methods that are more efficient and economical. This review thoroughly examines a variety of traditional and contemporary extraction techniques, their optimisation circumstances, and their relative benefits and drawbacks. Additionally, a huge variety of contemporary uses of these techniques have been evaluated critically. This literature review will aid in developing current extraction methods and identifying fresh ones.

2. Materials And Methods

Collection and authentication of Plant material

The fruit of *Foeniculum vulgare, Emblica officinalis* and aerial part of *Ocimum sanctum* were obtained from Haridwar city and authenticated by Department of Botany, IFTM University, Moradabad, U.P. Also, authentication done from Deendayal Research Institute, Arogyadham, Chitrakoot, Satna (M.P.).

Preparation of extracts

The plants were washed with distilled water to remove dirt and soil and shade dried in a ventilated place at room temperature. Each dried plant material was ground into a coarse powder individually using a mechanical mill, and then extracted using water as the solvent and a methanol: water (70:30) ratio in a soxhlet extractor for 18 hours. Filtered and concentrated under reduced pressure using rotavapo, the hydroalcoholic and aqueous extract of the fruit of *Foeniculum vulgare*, *Emblica officinalis*, and aerial part of *Ocimum sanctum* were then freeze-dried and kept in a deep freezer for later use. Each extract's solution was freshly made for each of the standardization parameters.

Physicochemical parameters

Physicochemical parameters like foreign matter, moisture content (loss on drying), total ash, water-soluble ash, acid-insoluble ash, sulphated-ash, alcohol-soluble extractive, water-soluble extractive and pH values were determined [9].

1. Determination of Foreign Matter:

The sample must be free of any unpleasant foreign matter, evident symptoms of mould growth, sliminess, bug contamination, and other animal and animal product products, including animal excrement. Any organism, component, or byproduct of an organism that is not listed in the specification of the product is considered foreign matter, as are mineral admixtures such soils, stones, sand, and dust. Beyond the bounds of the official parts of the organism, it must also comprise other parts. Unless otherwise stated, take 100 g of the sample and distribute it thinly on an appropriate platform. Examine in the light of day with an open mind or with a 6x or 10x magnification and separate the alien substance. The foreign stuff can also be separated using a suitable sieve. Sifting the sample through a 250-mesh screen separates the dust, which is thought to be a mineral admixture. Weigh the sorted foreign material and use the drug sample as a reference to determine the foreign matter content in percent.

2. Determination of Moisture Content (Loss on Drying):

Dry the evaporating dish for 30 minutes in the same environment that will be used to make the determination. In a tuned evaporating dish, add 5 to 10 g of carefully weighed powder or medication. Prepare 10 g of the sample for the unpowdered medication by cutting and shredding it into pieces they have a 3 mm thickness. Crack any seeds and fruits that are less than 3 mm. Avoid using high-speed mills to prepare the samples, and take care to ensure that the portion collected is an accurate representation of the official sample and that no considerable quantity of moisture is lost in the process. Shake the test object gently from side to side to a depth of about 5 mm, or no more than 10 mm in the case of bulky materials, to distribute the test object as evenly as possible. In the drying chamber, place the loaded bottle. Dry the test sample for three hours at 105°C, then weigh it. Continue drying and weighing every half an hour until the difference in weight between two subsequent measurements is no greater than 0.25 percent.

3. Determination of Total Ash:

About 2 to 3 g of the ground drug, precisely weighed, should be incinerated in a tared platinum or silica dish at a temperature no higher than 600°C until free from carbon. After cooling in a desiccator for 30 minutes, weigh the substance right away. In the event that this method is unsuccessful in producing

carbon-free ash, the charred mass can be put out with hot water, the residue collected on ashless filter paper, the residue and filter paper burned, the filtrate added, the residue burned to dryness, and the filtrate ignited at a temperature no higher than 600°C. Calculate how much ash there is in relation to the air-dried drug.

4. Determination of Water-Soluble Ash:

The ash from (2.4) should be boiled for five minutes with 25 ml of water; the insoluble material should be collected in a Gooch crucible or on ashless filter paper, washed in hot water, and ignited for fifteen minutes at a temperature no higher than 450 ºC. To determine the weight of the water-soluble ash, subtract the weight of the insoluble material from the weight of the ash. Determine the amount of water-soluble ash in relation to the air-dried medication.

5. Determination of Acid-insoluble Ash:

25 ml of diluted hydrochloric acid should be added dropwise to the crucible containing complete ash. Collect the insoluble material using Whatman 41 ashless filter paper, and then wash the filtrate in hot water until it is neutral. To ignite to a consistent weight, transfer the filter paper holding the insoluble material to the original crucible, dry on a hotplate, and ignite. After 30 minutes of cooling in a suitable desiccator, immediately weigh the residue. Determine the amount of acid-insoluble ash in the drug's air-dried form.

6. Determination of Sulphated Ash**:**

The silica crucible was heated for 10 minutes until it turned red, then it cooled in a desiccator before being weighed. Into the crucible was added 1 g of the powder. Lightly ignited at first, then continued until the material was completely burned. The residue was then cooled, wet with 1 ml of sulphuric acid, heated gradually until no longer emitting white fumes, and burned at $800^{o} \pm 25^{o}$C until no longer emitting any black particles. After allowing the crucible to cool, some sulfuric acid was added, and then it was heated. It was then lit as before and given time to cool. Without delay, the ash was weighed, and the percentage of sulphated ash in relation to air-dried powder was computed. The operation was repeated until two successive weighing do not differ by more than 0.5mg.

7. Determination of Alcohol-soluble Extractive:

In a closed flask, macerate 5 g of the air-dried medication in 100 ml of the specified alcohol strength for twenty-four hours, shaking frequently for the first six hours, and then letting stand for the last eighteen hours. In order to prevent solvent loss, filter the filtrate quickly. Then, evaporate 25 ml of the filtrate to dryness in a shallow dish with a flat bottom and dry it at 105 °C to a constant weight before weighing. Calculate the extractive's alcohol solubility percentage in relation to the air-dried medication.

8. Determination of Water-soluble Extractive:

Proceed as directed for the determination of Alcohol-soluble extractive, using chloroform water (2.5 ml chloroform in purified water to produce 1000 ml) instead of ethanol.

9. Determination of pH Values:

At the temperature the test material will be measured at, fill the cell with one of the Buffer Solutions for Standardization. Set the calibration control so that the observed pH value and the declared pH are the same, and then set the "temperature" control to the solution's temperature. Fill the cell with the second buffer solution for standardization after rinsing the electrodes with numerous parts of it at the same temperature as the substance being monitored. The second buffer solution's pH value is accurate to within ±0.07 pH units of the specified value. Examine the electrodes and replace them if there is a significant variation from the norm.

Preliminary Phytochemical Screening:

Phytochemical screening tests were carried out for the aqueous and hydroalcoholic extracts of Fennel, Amla and Tulsi for the presence or absence of various phytoconstituents [10].

1. Test for Carbohydrates:

Molish’s test: Take 2 to 3 ml of the extract, mix in a few drops of the alcohol-alpha-naphtol solution, and then condense H2SO4 off the test tube's sides. When two liquids come together, a violet ring forms.

2. Test for Amino acids:

Ninhydrin test: Boiling water bath for 10 minutes while heating 3 ml of extract and 3 drops of 5% Ninhydrin solution. Purple or bluish color appears.

3. Tests for Steroid:

(a) Salkowski reaction: 2 ml of extract, 2 ml of chloroform, and 2 ml of concentrated H2SO4 are used. Shake firmly. Acid layer exhibits fluorescence that is greenish yellow whereas the chloroform layer appears red.

(b) Liebermann-Burchard reaction: Combine chloroform and 2 ml of the extract. Add 2 drops of concentrated H2SO4 from the test tube's side and 1 to 2 ml of acetic anhydride. Colours first appear in red, then blue, and then green.

(c) Liebermann's reaction: 3 ml of the extract and 3 ml of the acetic anhydride should be combined. chilly and warm. H2SO4 solution, a few drops. The colour blue is seen.

4. Test for Fats and Oils:

(a) Solubility test: Oils are insoluble in 90% ethanol and water but soluble in ether, benzene, and chloroform.

(b) Saponification test: To get 10 ml of oil, evaporate the extract. Put in 25 cc of 10% NaOH. 30 minutes of boiling in a water bath. Cool. Boost the Na2SO4 solution amount. As it creates, soap rises to the top. Filter. Add H2SO4 to the filtrate. Evaporate. Gather the glycerol-containing residue. In ethanol, dissolve the residue. With ethanolic solution, perform following test:

(i) Take ethanolic solution, add few crystals of $KHSO_4$. Heat vigorously. Pungent odour of acrylic aldehyde was produced.

(ii) Take ethanolic solution and add few drops of $CuSO_4$ and NaOH solutions. Clear blue solution was observed.

5. Tests for Alkaloids:

Separately evaporate the aqueous and alcoholic extracts. Add diluted HCL to the residue. Filter after a good shake. Perform the following tests on the filtrate:

(a) Dragendorff's test: Add a few drops of Dragendorff's reagent to 2-3 ml of filtrates. The orange-brown powerpoint formed.

(b) Mayer's test: 2-3 ml of filtrates with a few drops of Mayer's reagent produce ppt.

(c) Hager's test: 1-2 ml filtrates with a few drops Yellow ppt is produced by Hager's reagent. (d) Wagner's test: 2-3 ml filtrates with a few drops Reddish-brown ppt is produced by Wagner's reagent.

6. Test for Tannins and Phenolic compounds:

Add a few drops of the following reagent to two to three ml of an aqueous or alcohol extract:

(a) Deep blue-black colour for the 5% FeCl3 solution.
(b) White ppt for lead acetate solution.
(c) White ppt for the gelatin solution.
(d) Bromine water: a change in colour.
(e) Red-colored acetic acid solution.
(f) Red ppt using potassium dichromate.
(g) Transient red colour in diluted iodine solution.

7. Test for Saponin glycosides:

Find out how much free sugar is in the extract. Add mineral acid to the extract to hydrolyze it. Find out once more how much sugar is in the hydrolyzed extract. The presence of glycoside in the extract is indicated by an increase in sugar concentration.

(a) Shake the medication extract or dry powder vigorously with water to see if foam forms. We saw persistent foam.

(b) Heamolytic test: Place one drop of blood on a glass slide and add a drug extract or dry powder. Hemolytic zone is visible.

8. Test for Flavonoids:

(a) The Shinoda test: Mix 0.5 g of magnesium turnings with 0.5 ml of 95% ethanol, a few drops of concentrated HCl, and dry powder or extract. Pink was spotted.

(b) Add lead acetate solution to a little amount of residue. The ppt. produced was yellow.

(c) The residue turns yellow as sodium hydroxide is added in increasing amounts.

9. Tests for Volatile oils:

Consider hydrodistillate substance. Perform the following tests after separating the volatile oil from the distillate:

(a) Volatile oils have a distinctive smell.
(b) Volatile oil does not leave a lasting stain on filter paper.
(c) Volatile oils are soluble in 90% alcohol, according to a solubility test.

10. Test for Heavy Metals:

Table no. 1: Permissible Limits of Heavy Metals

S. No.	Heavy Metal contents	Permissible limits
1	Lead	10 ppm
2	Arsenic	3 ppm
3	Cadmium	0.3 ppm
4	Mercury	1 ppm

Determination of Lead and Cadmium by Atomic Absorption Spectrophotometry or by Inductively Coupled Plasma:

Procedure: Prepare a test solution of the substance being examined as follows:

3 g of the test substance should be transferred to a dry, clean 300 ml Kjeldahl flask. [Note - If the reaction foams excessively, an 800-ml flask may be used]. Add enough strong nitric acid to completely wet the material and clamp the flask at a 45° angle. When the reaction starts, slowly heat the mixture until it subsides. Then, add little amounts of the same acid mixture, heating it again after each addition, until a total of 18 ml of acid has been added. Increase the heat and slowly boil the mixture until the solution turns black. After cooling, stir in 2 ml of nitric acid, then reheat the mixture until it turns dark. Nitric acid should then be added after continuing to boil the mixture until no further darkening occurs. Finally, the mixture should be heated vigorously to produce dense, white vapours. Cool carefully, add 5 ml of water, gradually boil until thick, white vapours begin to form, and then continue heating until only a few ml remains. After allowing it cool, carefully add 5 ml of water, and then check the solution's colour. If the hue is yellow, add 1 ml of 30% hydrogen peroxide slowly and let it evaporate again until it produces dense, white vapours that have a volume of 2 to 3 ml. Repeat the peroxide treatment and 5 ml of water addition if the solution is still yellow. Cool, cautiously dilute with a few milliliters of water, and rinse into a 50-milliliter color-comparison tube, being careful to ensure that the total volume does not exceed 25 millilitres. The same approach should be used to create a blank solution, but without the sample.

Prepare a minimum of three standard solutions of the element being tested, each at a different concentration, to cover the possible range of 25 to 200 percent in the sample solution. The corresponding reagents for the test solution should be added separately, and the corresponding reagents should also be used to generate the blank reference solution. Set the analytical conditions appropriate for the analysis of lead and cadmium, calibrate the instruments, and run them in accordance with the manufacturer's instructions.

Record your measurements of the absorbances of the blank reference and each reference solution at a different concentration, then use the average value of three readings for each concentration on the ordinate and the corresponding concentration on the abscissa to construct a calibration curve.

By extrapolating the mean value of the values made using the test solution on the calibration curve, determine the concentration of each heavy metal.

Tests for Aflatoxin:

Aflatoxins are extremely toxic, so handling aflatoxin compounds requires great caution. Aflatoxin B1, B2, G1, and G2 may be detected with this test in any material derived from plants. Use the procedure below unless the individual monograph specifies differently.

Zinc Acetate - Aluminum Chloride Reagent: Dissolve 20 g of zinc acetate and 5 g of aluminum chloride in sufficient water to make 100 ml.

Sodium Chloride Solution: Dissolve 5 g of sodium chloride in 50 ml of purified water.

Test Solution 1: Transfer 5 g or so of the powdered substance, precisely weighed, to a flask with a glass stopper. 200 ml of a 17:3 methanol and water mixture should be added. Shake ferociously using mechanical means for at least 30 minutes before filtering. [Note - Follow the instructions for Test Solution 2 if the solution contains interfering plant pigments. Collect the remaining 40 ml of the filtrate after discarding the first 50 ml. To a separating funnel, transfer the filtrate. Shake for one minute after adding 40 ml of sodium chloride solution and 25 ml of hexane. Transfer the bottom aqueous layer to a second separating funnel after allowing the layers to separate. Shaking the aqueous layer in the separating funnel for one minute after twice extracting it with 25 ml of methylene chloride each time. Every time, let the layers separate, then remove the solvent from the mixed layers and let them evaporate over a water bath. Let the residue cool. If there are any interferences in the residue, follow the cleanup procedure instructions; if not, dissolve the residue in 0.2 ml of a 9.8:0.2 solution of chloroform and acetonitrile and shake it with a mechanical device, if necessary.

Test Solution 2: 100 ml of the filtrate from the beginning of the flow should be collected and transferred to a 250 ml beaker. 80 ml of water and 20 ml of the zinc acetate-aluminum chloride reagent should be added. Stir and let stand for five minutes. Filter after adding 5 g of a suitable filtering aid, such as diatomaceous earth. Collect the remaining 80 ml of the filtrate after discarding the first 50 ml. Start by "Transfer the filtrate to a separating funnel" as you would for Test Solution 1, then proceed as instructed.

Cleanup Procedure: In a 10 mm x 300 mm chromatographic tube, insert a glass wool plug or a medium-porosity sintered-glass disc. Diethyl ether and hexane mixed in a 3:1 ratio are used to make a slurry out of 2 g of silica gel, which is then poured into a column and washed with 5 ml of the same solvent combination. Allow the absorbent to settle before adding 1.5 g of anhydrous sodium sulphate to the column's top. The above-mentioned residue should be dissolved in 3 ml of methylene chloride before being added to the column. The flask should be rinsed twice with 1 ml volumes of methylene chloride. The rinses should then be added to the column and eluted at a rate of no more than 1 ml per minute. Hexane, diethyl ether, and methylene chloride should be added to the column in that order. Then, the column should be eluted at a rate of no more than 3 ml per minute, and the eluates should be discarded. Add 6 ml of a 9:1 methylene chloride and acetone solution to the column, and then elute at a rate not exceeding 1 ml per minute, ideally without the use of vacuum. Collect this eluate in a tiny vial, if necessary, add a boiling chip, and let it dry on a water bath. The residue should be dissolved in 2 ml of a 9.8:0.2 solution of chloroform and acetonitrile and shaken if necessary. **Aflatoxin Solution:** A solution with a concentration of 1.0 g/ml for aflatoxins B1 and G1 and 0.2 g/ml for aflatoxins B2 and G2 can be made by dissolving precisely weighed amounts of aflatoxins B1, B2, G1, and B2 in a mixture of chloroform and acetonitrile (9.8:0.2).

Procedure: Apply three separate 10 µl applications of either Test Solution 1 or Test Solution 2 on a suitable thin-layer chromatographic plate that has been covered with a 0.25 mm layer of chromatographic silica gel. Separately apply 2.5, 5, 7.5, and 10 µl of the Aflatoxin Solution. Apply 5 µl of the Aflatoxin Solution over one of the Test Solution's three 10 µl applications. After allowing the spots to dry, develop the chromatogram in an unsaturated chamber with a solvent system made up of an 85:10:5 ratio of isopropyl alcohol, chloroform, and acetone until the solvent front has migrated at least 8 cm from the origin. Mark the solvent front of the plate after removing it from the developing chamber and letting it air dry. Examine the plate under UV light at 366 nm to find the spots: the four applications of the Aflatoxin Solution appear as four distinct blue fluorescent spots; the spot from the Test Solution superimposed on the Aflatoxin Solution is not more intense than the corresponding spot from the Aflatoxin Solution; and none of the other Test Solutions correspond to any of the applications of the Aflatoxin Solution. If any Aflatoxin are found in the Test Solution, the kind of Aflatoxin present will be determined by matching the colour of each fluorescent spot's position with that of the Aflatoxin Solution. The estimated concentration of Aflatoxin in the Test Solution can be determined by comparing the intensity of the Aflatoxin spot, if present, with that of the corresponding Aflatoxin in the Aflatoxin Solution.

Spray gas: Inert gas (Nitrogen)
Sample solvent type: Methanol
Dosage speed: 150 nl/s
Predosage volume: 0.2 ul

Table no.2: Aflatoxin profile at 254nm & 366nm:

Solvent system	Chloroform:acetone isopropyl:alcohol (85:10:5)
Distance travelled by solvent	8cm

Stationary phase	TLC precoated plate with silica gel 60F254 plate of 0.2mm thickness
Test Solution	10µl
Developement Chamber	twin trough chamber (20*10 with SS lid)
Visiualization	254nm & 366nm; after development

Table no.3: Sample preparation

S. No.	Appl. Position	Appl. volume	Vial	Sample ID	Active
1.	15.0 mm	10.0 µl	1	Aflatoxin Mix	Yes
2.	36.2 mm	10.0 µl	2	Amla Aqueous	Yes
3.	57.4 mm	10.0 µl	3	Amla Hydroalcoholic	Yes
4.	78.6 mm	10.0 µl	1	Aflatoxin Mix	Yes
5.	99.8 mm	10.0 µl	4	Tulsi Aqueous	Yes
6.	121.0 mm	10.0 µl	5	Tulsi Hydroalcoholic	Yes
7.	142.2 mm	10.0 µl	1	Aflatoxin Mix	Yes
8.	163.4 mm	10.0 µl	6	Fennel Aqueous	Yes
9.	184.6 mm	10.0 µl	7	Fennel Hydroalcoholic	Yes

Thin-layer chromatography:

Fennel (aqueous and hydroalcoholic extract): Utilizing aqueous and hydroalcoholic extract as the sample solution, thin-layer chromatography was performed on a precoated silica gel 60F254 plate.

Test solution: Use maceration procedures with 100ml alcohol and 10g of coarsely powdered substance in a 250ml stoppered conical flask for a 24-hour extraction. Shake occasionally. To create up to 100ml in a volumetric flask, decant the extract.

Solvent system: toluene ethyl acetate (9.0:1.0).

Procedure: Apply 10 µl of each of the hydroalcoholic and aqueous extract solutions in bands of 10 mm on a TLC plate. Develop the plate until it is 8 cm away from the application line. Examine the plate under 254 nm after air-drying it. Anisaldehyde sulphuric acid reagent solution should be sprayed onto the plate. For about 5 minutes, or until the bands are clearly visible, heat the plate at 110 degrees. The chromatogram produced by combining aqueous and hydroalcoholic solutions reveals bands at 254 nm and 366 nm that correspond to their constituents and the TLC profile.

Amla (aqueous and hydroalcoholic extract): Utilising aqueous and hydroalcoholic extract as the sample solution, thin-layer chromatography was performed on a precoated silica gel 60F254.

Test solution: Add 25 ml of methanol to 0.2g of the drug being tested from the aqueous and hydroalcoholic extract, heat on a water bath for 10 to 15 minutes, cool, and filter.

Solvent system: toluene: ethyl acetate: formic acid (2.0:5.0:1.5).

Procedure: Apply 10 µl of the hydroalcoholic and aqueous extract solutions in bands of 10 mm on a TLC plate. Develop the plate until it is 8 cm away from the application line. Examine the plate under 254 nm after air-drying it. Anisaldehyde sulphuric acid reagent solution should be sprayed onto the plate. For about 5 minutes, or until the bands are clearly visible, heat the plate at 110 degrees. The chromatogram produced by combining aqueous and hydroalcoholic solutions reveals bands at 254 nm and 366 nm that correspond to their constituents and the TLC profile.

Tulsi (aqueous and hydroalcoholic extract): Utilising aqueous and hydroalcoholic extract as the sample solution, thin-layer chromatography was performed on a precoated silica gel 60F254 plate.

Test solution: By refluxing 1 g of the material as a sample solution for 15 minutes with 50 ml of methanol, it can be extracted from aqueous and hydroalcoholic extracts. Concentrate the extract to 25 ml by filtration.

Solvent system: toluene: ethyl acetate: acetic acid (8.0:2.0:0.1).

Procedure: Apply 10 µl of the hydroalcoholic and aqueous extract solutions in bands of 10 mm on a TLC plate. Develop the plate until it is 8 cm away from the application line. Examine the plate under 254 nm after air-drying it. Anisaldehyde sulphuric acid reagent solution should be sprayed onto the plate. For about 5 minutes, or until the bands are clearly visible, heat the plate at 110 degrees. The chromatogram produced by combining aqueous and alcoholic hydrosolutions displays bands at 254 nm and 366 nm that correspond to their constituents and the TLC profile [11].

Rf Value: $\text{Rf value} = \frac{\text{Distance moved by solute}}{\text{Distance moved by solvent}}$

3. Results and Discussion

Physicochemical parameters

Physicochemical parameters like foreign matter, moisture content (loss on drying), total ash, water-soluble ash, acid-insoluble ash, sulphated-ash, alcohol-soluble extractive, water-soluble extractive and pH values were showed following results shown in table no.5.

Table no. 4: Permissible Limits of physicochemical parameters:

Quantitative Parameters	Fennel Aqueous Extract	Fennel Hydroalcoholic Extract	Amla Aqueous Extract	Amla Hydroalcoholic Extract	Tulsi Aqueous Extract	Tulsi Hydroalcoholic Extract
Loss on drying	NMT 7.0 %	NMT 7.0 %	NMT 7.0 %	NMT 5.0 %	NMT 5.0 %	NMT 5.0 %
Total ash	NMT12.0 %	NMT12.0 %	NMT10.0 %	NMT10.0 %	NMT10.0 %	NMT12.0 %
Acid-insoluble ash	NMT15.0 %	NMT15.0 %	NMT 1.25 %	NMT 1.5 %	NMT 1.0 %	NMT 1.5 %
pH	3.5-5.5	3.5-5.5	LT 3.5	3.0 - 4.5	3.5-5.5	3.5-5.5
Total soluble solids	NLT 90.0 %	NLT 90.0 %	NLT 90.0 %	NLT 90.0 %	NLT 90.0 %	NLT 90.0 %
Heavy metals	Complies with the prescribed limits	Complies with the prescribed limits	Complies with the prescribed limits	Complies with the prescribed limits	Complies with the prescribed limits	Complies with the prescribed limits
Reference	API VOL-1, 1986	API VOL-1, 1986	API VOL-8, 2010	API VOL-8, 2010	API VOL-9, 2016	API VOL-9, 2016

Table no.5: Pharmacognostical Studies:

S.No.	Analytical Parameters	Fennel (%w/w)			Amla (%w/w)			Tulsi (%w/w)		
	FRACTIONS	**RM**	**AQ.**	**ALC**	**RM**	**AQ.**	**ALC**	**RM**	**AQ.**	**ALC**
1.	Foreign matter	1.7	-	-	1.9	-	-	1.5	-	-
2.	Moisture Content (Loss on Drying)	7.88	3.45	3.67	7.66	3.67	3.01	10.09	3.69	3.72
3.	Total Ash	4.56	5.98	4.66	2.32	3.92	2.59	9.46	9.29	9.76
4.	Acid-Insoluble Ash	1.22	0.78	0.85	1.66	0.48	0.94	2.69	0.89	0.99
5.	Water -Soluble Ash	0.46	0.73	0.81	0.98	0.64	0.92	4.62	0.90	1.21
6.	Sulphated Ash	3.66	3.12	3.25	2.23	2.86	2.46	7.23	9.01	9.52
7.	Alcoholic soluble extractive value (70:30)	10.05	53.56	67.88	41.16	49.91	66.55	8.00	31.78	46.49
8.	Water soluble extractive value	7.98	78.31	84.34	54.70	33.65	58.04	14.24	84.62	79.39
9.	PH	-	5.21	5.38	-	3.43	3.86	-	4.97	5.13

Preliminary Phytochemical Screening:

Phytochemical screening tests were carried out for the aqueous and hydroalcoholic extracts of fennel, Amla and Tulsi for the presence or absence of various Phytoconstituents. All Phytoconstituents were showed following results shown in table no.6 & 7.

Table no.6: Preliminary Phytochemical Screening of Herbal Plants:

S.No.	Phytochemical screening	FENNEL		AMLA		TULSI	
	Extract	**Aq.**	**Alc.**	**Aq.**	**Alc.**	**Aq.**	**Alc.**
1.	Carbohydrates	+ve	+ve	+ve	+ve	-ve	-ve
2.	Amino Acids	-ve	-ve	+ve	+ve	-ve	-ve
3.	Steroid	-ve	+ve	-ve	+ve	+ve	+ve
4.	Fats and Oils	-ve	+ve	-ve	-ve	-ve	+ve
5.	Alkaloids	-ve	+ve	-ve	-ve	+ve	+ve
6.	Tannins and Phenoilc compounds	+ve	+ve	+ve	+ve	+ve	+ve
7.	Saponin Glycoside	+ve	-ve	-ve	-ve	+ve	+ve
8.	Flavonoid	+ve	+ve	+ve	+ve	+ve	+ve
9.	Volatile oils	+ve	+ve	+ve	+ve	+ve	+ve

+ve = Present **-ve = Absent** **Aq. = Aqueous** **Alc. = Hydroalcoholic**

Table no. 7: Test for Heavy Metals

S. No.	Heavy Metal Contents	Fennel (ppm)		Amla (ppm)		Tulsi (ppm)		Permissible Limits
	Extract	Aq.	Alc.	Aq.	Alc.	Aq.	Alc.	ppm
1	Lead	0.00604	0.006134	0.00349	0.00300	0.004767	0.007293	10
2	Cadmium	0.00309	0.002295	0.00364	0.00211	0.003216	0.001895	0.3

Test for Aflatoxins: Aflatoxins are extremely toxic, so handling aflatoxin compounds requires great caution. Aflatoxin B1, B2, G1, and G2 may be detected with this test in any material derived from plants. Results were showed absence of Aflatoxins in all three phytoconstituents (Amla, Tulsi and fennel) in TLC report at 254nm and 366nm which shown in fig no.1 & 2 respectively.

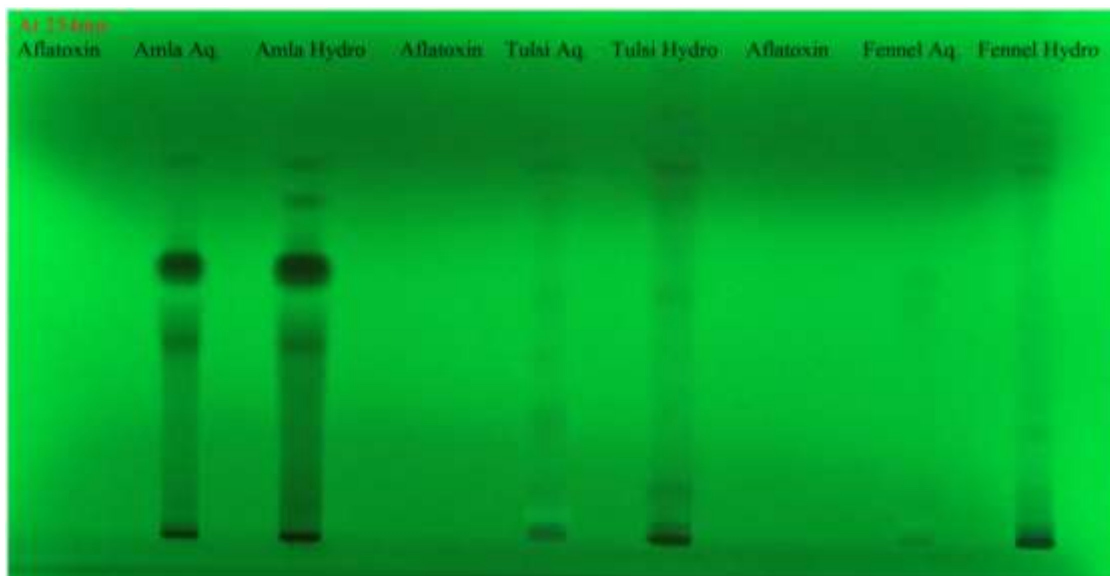

Fig. no.1: Spots of Aflatoxin and plant extracts on TLC plate at 254nm

Fig. no. 2: Spots of Aflatoxin and plant extracts on TLC plate at 366nm

Thin-layer chromatography:

Thin-layer chromatography carried out on a precoated silica gel 60F254 plate using aqueous and hydroalcoholic extract as sample solution. The results were shown in fig no.3, 4 & 5.

Fennel (aqueous and hydroalcoholic extract):

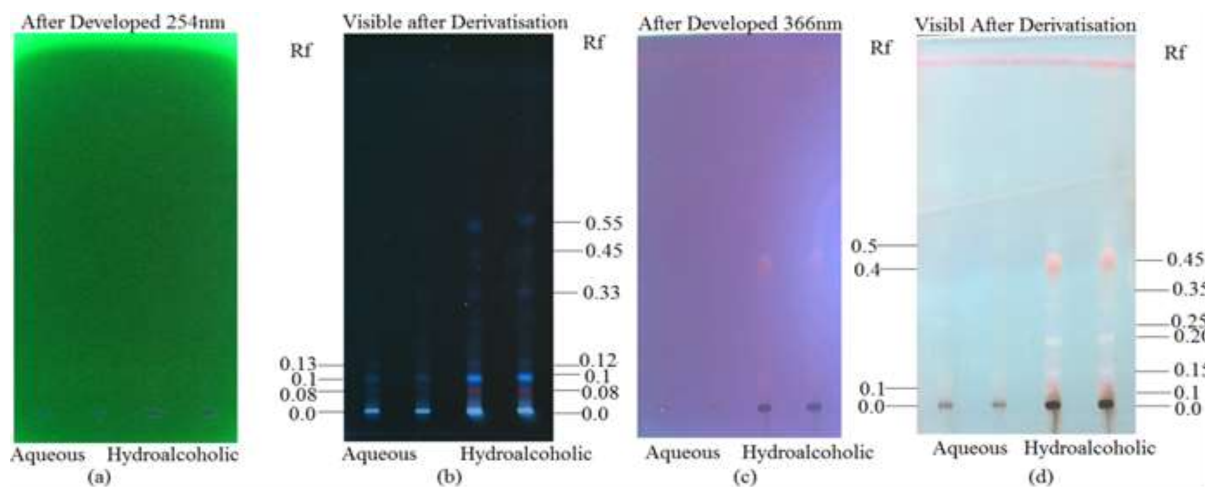

Fig. no. 3: Thin-Layer Chromatogram of Fennel aqueous and hydroalcoholic extract. (a) & (b) at 254nm, (c) & (d) at 366nm

Table no.8: TLC profile of Fennel (aqueous and hydroalcoholic extract) at 254nm & 366nm:

Solvent system	Toluene:ethyl acetate (9.0 :1.0).							
Distance travelled by solvent	8cm							
Stationary phase	TLC precoated plate with silica gel 60F254 plate of 0.2mm thickness							
Test Solution	10µl							
Developement Chamber	twin trough chamber (20*10 with SS lid)							
Visiulaization	254nm & 366nm; after derivatisation with Aniseldehyde-Sulphuric acid							
Extract	**Hydroalcoholic Extract (10 µ)**				**Aqueous Extract (10 µ)**			
Total No. of spots	**14**				**8**			
Range	**At 254nm (7)**		**At 366nm (7)**		**At 254nm (4)**		**At 366nm (4)**	
Observation	**Rf value**	**Colour of spot**	**Rf value**	**Colour of spot**	**Rf value**	**Colour of spot**	**Rf value**	**Colour of spot**
	(0.0) (0.08) (0.1) (0.12) (0.33) (0.45) (0.55)	Sky blue Reddish blue Dark blue Blue Light blue Dark blue Blackish blue	(0.0) (0.1) (0.15) (0.20) (0.25) (0.35) (0.45)	Brown Pink Peach Cream Light purple Purplish blue Dark peach.	(0.0) (0.08) (0.1) (0.13)	Sky blue purplish blue dark blue blackish blue	(0.0) (0.1) (0.4) (0.5)	Light brown Light peach Pink Light yellow.

Amla (aqueous and hydroalcoholic extract):

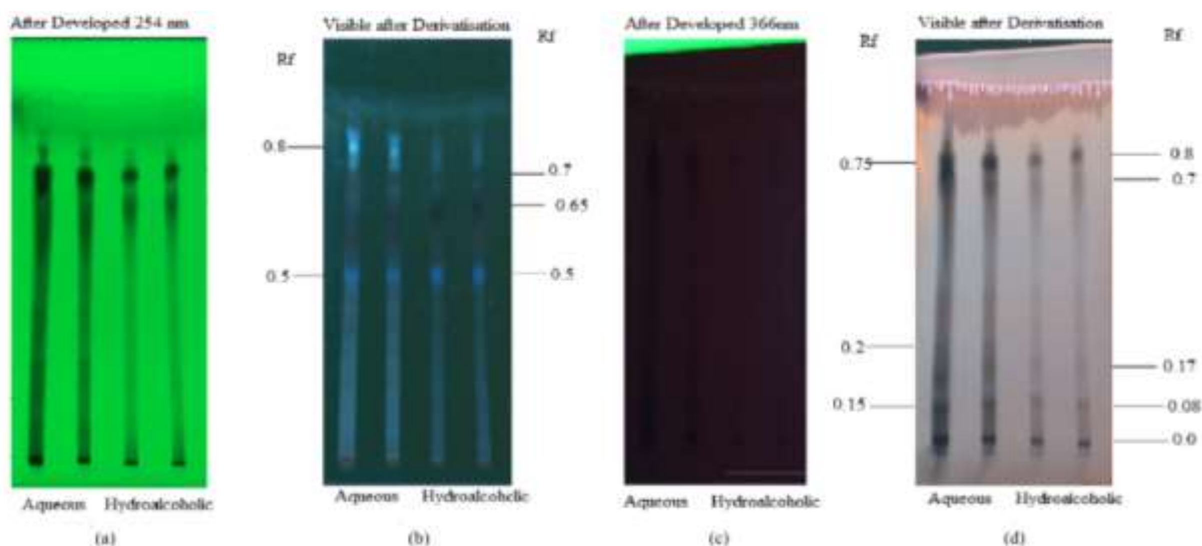

Fig. no. 4: Thin-Layer Chromatogram of Amla aqueous and hydroalcoholic extract (a) & (b) at 254nm, (c) & (d) at 366nm

Table no. 9: TLC profile of Amla (aqueous and hydroalcoholic extract) at 254nm & 366nm:

Solvent system	(Toluene : ethyl acetate : formic acid (2.0 : 5.0 : 1.5)							
Distance travelled by solvent	8cm							
Stationery phase	TLC precoated plate with silica gel 60F254 plate of 0.2mm thickness							
Test Solution	10µl							
Developement Chamber	twin trough chamber (20*10 with SS lid)							
Visiulaization	254nm & 366nm; after derivatisation with Aniseldehyde-Sulphuric acid							
Extract	**Hydroalcoholic Extract (10 µ)**				**Aqueous Extract (10 µ)**			
Total No. of spots	**8**				**5**			
Range	**At 254nm (3)**		**At 366nm (5)**		**At 254nm (2)**		**At 366nm (3)**	
Observation	**Rf value**	**Colour of spot**	**Rf value**	**Colour of spot**	**Rf value**	**Colour of spot**	**Rf value**	**Colour of spot**
	(0.5) (0.65) (0.7)	Light blue Blackish blue Dark blue.	(0.0) (0.08) (0.17) (0.7) (0.8)	Black Yellow Grey Dark grey Black	(0.5) (0.8)	Blue Bright blue	(0.15) (0.2) (0.75)	Yellowish green Dark yellow Black.

Tulsi (aqueous and hydroalcoholic extract):

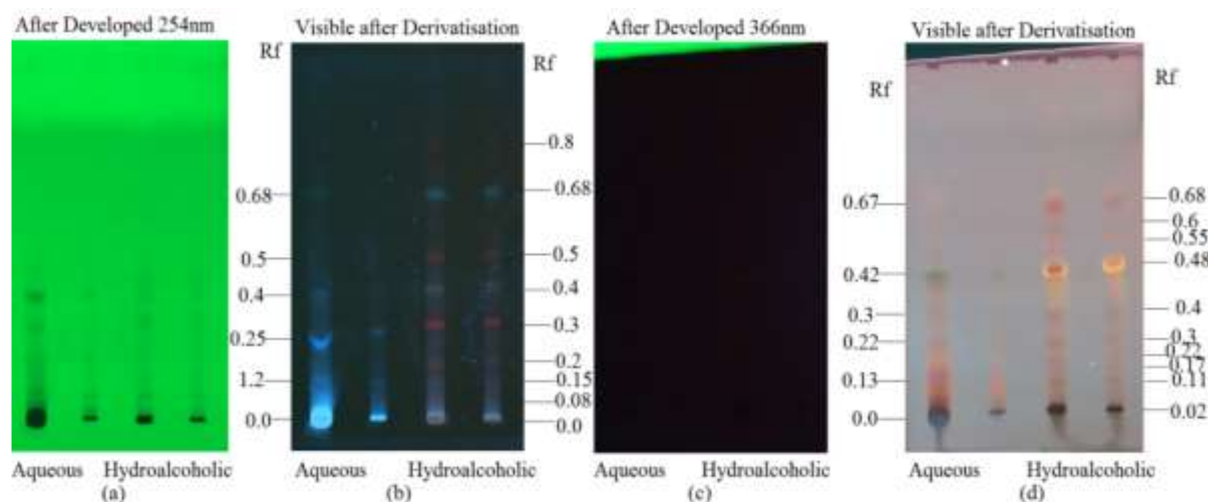

Fig. no.5: Thin-Layer Chromatogram of Tulsi aqueous and hydroalcoholic extract (a) & (b) at 254nm, (c) & (d) at 366nm

Table no. 10: TLC profile of Tulsi (aqueous and hydroalcoholic extract) at 254nm & 366nm:

Solvent system	toluene: ethyl acetate: acetic acid (8.0 : 2.0 : 0.1)							
Distance travelled by solvent	8cm							
Stationary phase	TLC precoated plate with silica gel 60F254 plate of 0.2mm thickness							
Test Solution	10µl							
Developement Chamber	twin trough chamber (20*10 with SS lid)							
Visiulaization	254nm & 366nm; after derivatisation with Aniseldehyde-Sulphuric acid							
Extract	**Hydroalcoholic Extract (10 µ)**				**Aqueous Extract (10 µ)**			
Total No. of spots	**19**				**12**			
Range	**At 254nm (9)**		**At 366nm (10)**		**At 254nm (6)**		**At 366nm (6)**	
Observation	**Rf value**	**Colour of spot**	**Rf value**	**Colour of spot**	**Rf value**	**Colour of spot**	**Rf value**	**Colour of spot**

	(0.0) (0.08) (0.15) (0.2) (0.3) (0.4) (0.5) (0.68) (0.8)	Reddish blue Blueish black Blue Reddish Bright red Purple Red Sky blue Red	(0.02) (0.11) (0.17) (0.22) (0.3) (0.4) (0.48) (0.55) (0.6) (0.68)	Black Orange Pink Purple Light brown Yellow Bright yellow Pink Purple Peach	(0.0) (0.12) (0.25) (0.4) (0.5) (0.68)	Bright blue Blue Bright blue Blackish value Whitish grey Bluish grey	(0.0) (0.13) (0.22) (0.3) (0.42) (0.67)	Bluish black Pinkish purple Orange Grey Black Peach

4. Conclusion

In this research article we concluded that all Phytoconstituents extracted from Fennel, Amla and Tulsi were non-toxic and safe. All Phytoconstituents were free from heavy metals and Aflatoxin. These can be utilized to create polyherbal formulations, which are receiving a lot of attention from researchers studying natural products around the world. A crucial stage in the investigation of natural products is the extraction of bioactive substances. It has been the obstacle to screening ever-greater numbers of items quickly. Currently, extraction entails separating the molecular components of plant tissues that are medicinally active from the inert components utilizing conventional solvent extraction techniques or the common modern and environmentally friendly extraction methods. The selection of the extraction method is essential since it affects the accuracy and caliber of the subsequent analytical processes. The major goals of extraction are to increase yields of bioactive substances, reduce extraction time, be more environmentally friendly, and attain economic viability without sacrificing biological activity. Therefore, the use of these herbal plants in formulating polyherbal formulations would surely help in treatment of various neurological disorders. A detailed, discussed and planned researches in this area would prove to be a new trust in line of therapeutics.

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Conflicts Of Interest

All authors declare that they have no conflicts of interest.

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