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ORIGINAL ARTICLE **OPEN ACCESS**

Physicochemical Standardization and evaluation of *Nigella sativa*, *Moringa olifera* and *Curcuma longa*

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ABSTRACT
The use of medicinal plants in both crude and prepared forms has increased greatly. The world health organization has estimated that 80% of the global population relies chiefly on traditional medicine for health care and there are reports that about 51% of all drug preparations in industrialized countries derive from plants, acting as sources of therapeutic agents or models for new synthetic compounds, or as raw material for semisynthetic production of highly complex molecules. The main objective of the proposed study is to formulate and evaluate a herbal dosage form containing extract of plant named Nigella sativa, Moringa olifera and Curcuma longa. The plants will be collected and authenticated. They will be extracted successive solvent extraction followed by preliminary phytochemical evaluation of all the extracts. The extract with potential chemical constituents will be selected for fractionation with various solvents. The traditional literature claims numerous medicinal plants having anti inflammatory potential, however the lack of research studies on these plants make their application in the pharmaceutical industry very insignificant. A number of physicochemical characteristics were calculated, including swelling index, ash content, acid insoluble ash, and water soluble ash extractive value. Nigella sativa, Moringa olifera, and Curcuma longa medication extracts were reported to have ash content values of 7.20 % w/w, 7.13% w/w, 7.06% w/w, and 7.05% w/w, respectively. While Nigella sativa, Moringa olifera, and Curcuma longa medicine extract were found to have water soluble ash contents of 3.92%, 3.94%, 4.23%, and 4.61%, respectively. The yield of the aqueous extract with semisolid masses of brownish, greenish, and bright yellow colours, respectively, was determined to be 6.37, 7.83, and 4.23% w/w of the crude drug. Further studies are required to check the anti inflammatory potential and other pharmacological activity of these plants.
Keywords: *Nigella sativa, Moringa olifera, Curcuma longa, Herbal plants, Physicochemical Standardization*

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INTRODUCTION

Herbal medicine is still widely used as a drug or an alternative medicine to treat diseases. Herbal medicines are naturally occurring plant-derived substances used for treatment or medicinal purposes [1]. To their definition, herbal medicine should not contain a synthetic chemical or medicinal isolation result. Since the market for herbal medicine is growing up continuously, there are reports that herbal medicine contains an undeclared synthetic drug to increase the therapeutic effect [2]. One of the undeclared synthetic drugs in herbal medicine is dexamethasone.[3,4]

Herbal medicines usually contain a range of pharmacologically active compounds; in some cases it is not known which ingredients are important for the therapeutic effect. Many herbalists believe that isolated ingredients have weaker clinical effects than whole plant extracts, a notion that would obviously require proof in each case. The multi-ingredient character of herbal medicines can render efficacy testing more complex than with synthetic drugs. One approach is to view the entire herbal extract as the active principle. To optimize the reproducibility of such studies, extracts need to be sufficiently characterized. This is often attempted through standardization according to a key constituent of the extract (e.g. a pharmacologically active ingredient or, if such an ingredient into known, a marker suitable substance). Standardization can, however, only cover one or two ingredients; thus variation of other ingredients may still remain, and it is possible that this could influence both efficacy and safety of the product. Full product characterization and quality control are therefore essential for the reproducibility of scientific tests of herbal remedies.[5]

Medicinal plants frequently include a range of pharmacologically active compounds; occasionally, the precise ingredients required for the therapeutic activity are unclear. Although this assertion would need to be supported in each case, many herbalists believe that isolated components have fewer medicinal benefits than whole plant extracts. Effectiveness testing for herbal medicines may be more challenging than for manufactured pharmaceuticals due to their multi-ingredient nature. One approach is to think of the whole herbal extract as the active component. In order to increase the reproducibility of such study, extracts must be well characterised. Standardizing the extract based on a significant constituent is a common attempt at doing this (e.g. a pharmacologically active ingredient or, if such an ingredient is not known, a marker suitable substance).

The plant kingdom is the source of many of today's manufactured medications, and only 200 years ago, herbal cures predominated our pharmacopoeia. Medical herbalism—the use of drugs that are made entirely of plant material—saw a swift decline when pharmacology became the preeminent field of therapeutics. Around a century ago, herbal medicine mostly disappeared from the therapeutic landscape in the English-speaking world. On the other hand, medical herbalism was never completely abandoned in many developing nations (such as Ayurvedic medicine in India, Kampo medicine in Japan, and Chinese herbalism in China), and it coexisted with modern pharmacology in some nations, like Germany and France, albeit to a lesser extent. In recent years, the situation has started to change once more. 16.4% of all patients attending an internal medicine clinic now use herbal drugs, according to a more recent US survey.[6,7]

MATERIAL AND METHODS

Collection, Identification and Authentication of Plant Specimens

The medicinal properties of plants have been investigated in the light of recent scientific developments throughout the world, due to their potent pharmacological activities, low toxicity and economic viability. As per the information collected from local vaidhyas and other traditional anti-inflammatory medicine practitioners, the present study is deals with the evaluation of activity of *Nigella sativa, Moringa oleifera* and *Curcuma Longa* .

Preparation of Extract

Procured locally, authenticated by Department of Botany, by the studies include organoleptic tests, macroscopic and microscopic observations. They were washed twice using tap water and then washed again in distilled water to remove the dust. The different parts were dried in the shade for 7–12 days at room temperature, until they were free from the moisture and then pulverized into coarse powder. The powdered seeds was added separately in soxhlet tube, methanol was also added to both soxhlet tube and round bottom flask, it was subjected to continuous hot extraction (soxhlet) with methanol at 50-60° Celsius for 16 to 20hrs. Ethanol for ethanolic extract, water for aqueous extract and water and alcohol in a ratio of 50:50 for hydroalcoholic extract respectively. The complete extraction was confirmed by taking about 5 ml solvent from the thimble and evaporated to check for the absence of residue and solvent in siphon was colorless. The extracts were concentrated using Rotary evaporator under reduced pressure below 40°C to get reddish-brown semi solid extract. The obtained mass for each crude drug was weighed, subjected for lyophilized to get free from methanolic solvent and kept in vacuum desiccators. Procedure was repeated to get sufficient amount of extract. Weight of round bottom flask with extract was measured every time and noted for calculation of percentage of yield. Later it was transferred into a container and the filtrate was vacuum concentrated to remove the moisture content. Finally the extract was stored at -4°C..

Principle editing

The fat extractor uses the solvent reflux and siphon principle to continuously extract the solid matter by pure solvent, which saves the solvent extraction efficiency and high efficiency. The solid material is ground prior to extraction to increase the area of solid-liquid contact. The solid material is then placed in a filter paper holder and placed in an extractor. The bottom end of the extractor is connected to a round bottom flask containing a solvent, and is connected to a reflux condenser. The bottom flask is heated to boil the solvent, the vapor rises through the branch pipe of the extractor, is condensed and drops into the extractor, and the solvent is contacted with the solid for extraction. When the solvent surface exceeds the highest point of the siphon, the solvent containing the extract is siphoned back. The flask, thus extracting a portion of the material, is repeated such that the solid material is continuously taken as a pure solvent and the extracted material is concentrated in the flask.

Since the extract of the organic solvent contains more or less fats such as free fatty acids, sterols, phospholipids, waxes, and pigments, the results of the Soxhlet extraction method can only be crude fat.

Determination of Extractive Values

Extractive values are used for evaluation of crude drugs when they cannot be estimated by any other method. Extractive values by different solvents are used to assess quality, purity and to detect adulteration due to exhausted and incorrectly processed drugs. Crude drugs contain a number of constituents and these have a selective solubility in different solvents. Water, alcohol, alcohol/water mixtures, generally 45%, 60%, 90% ethanol, ether are used as solvents to prepare ethanol soluble extractive, water soluble extractive (chloroform water), ether soluble extractive, etc. Extractive values indicate the presence of different constituents and TLC fingerprints can be developed for identification and semi-quantitative analysis from these extracts.

5 g seed powder of all the study samples were extracted separately with 100 ml solvent (Methanol, Petroleun Ether, Chloroform, water separately) in a closed flask for 24 hours. The flask was intermittently shaken during the first 6 hours and allowed to settle without any movement / disturbance for 18 hours. The extract was filtered with precaution avoiding solvent loss and 25 ml of the filtrate was evaporated to dryness in a dried, weighed, tared flat bottom evaporating dish and dried at 105°C to a constant weight. From the weight of the residue obtained, the percentage of alcohol soluble extractive was calculated in accordance to the air-dried drug and then percentage of alcohol soluble extract was calculated using the following formula:

Percentage yield of extract = (Weight of residue x100x100/ weight of sample x25) % w/w.

Standardization parameters

The different standardization/physicochemical methods were carried out in dried powder of different Extracts of *Nigella sativa, Moringa olifera and Curcuma longa*.

Foreign Matter

Anything extra present in the drug which does not comply with the authentic drug may be considered as a foreign matter. The foreign matter can be present in the drug due to improper harvesting. The source of foreign organic matter can be animal excreta, insect, or mold and is determined by sedimentation or floatation method. Herbal drugs should be made from the stated part of the plant and be devoid of other parts of the same plant or other plants. They should be entirely free from moulds or insects, including excreta and visible contaminant such as sand and stones, poisonous and harmful foreign matter and chemical residues. Animal matter such as insects and "invisible" microbial contaminants, which can produce toxins, are also among the potential contaminants of herbal medicines. Macroscopic examination can easily be employed to determine the presence of foreign matter, although microscopy is indispensable in certain special cases (for example, starch deliberately added to "dilute" the plant material). Furthermore, when foreign matter consists, for example, of a chemical residue, TLC is often needed to detect the contaminants.

Weigh 100gm of the powder drug to be examined or the minimum quantity prescribed in the monograph and spread it out in a thin layer. With the help of eye or by the use of lens (6 xs) we can detect the foreign matter. Separate and weigh it and calculate the percentage present.

- 100 g of sample was weighed.
- The sample was spread on a white tile or glass plate uniformly without overlapping.
- Inspected the sample with naked eyes and by means of a lens (5x or above).
- The foreign matter (mentioned above) was separated manually.
- After complete separation, the foreign matter was weighed and determined percent weight by weight (% w/w) present in the sample.
- Percentage of foreign matter was calculated using the following formula. For which gross weight of the sample was noted

% Foreign Matter = W1x 100/ W2

Where,

W= Weight of the sample.

W1= amount of Foreign Matter.

W2 = amount of drug taken.

Total Ash value

After the incineration of the crude drugs, the remnants are known as ash. Ash contains mostly inorganic salts. It gives the idea about the quality and purity of crude drugs. It can be determined by (a) Total ash: Carbon and organic matters of the crude drugs are converted to ash at a temperature of about 900°C. It mostly contains carbohydrates, phosphates, silicates, and silica. This total ash is further used with water-soluble ash and acid-insoluble ash. Water-soluble ash is produced by separating the water-soluble materials, which is dried to yield water-soluble ash. Further, total ash, when treated with dilute hydrochloric acid, removes many inorganic salts to yield mainly silica in the residue. This is known as acid-insoluble ash.

Ash was calculated by weighing 2 gm of a dried crude drug sample in a crucible. It was then heated using a hot plate. The residue of carbon was obtained as a result of ignition when placed in a muffle furnace at 500°C till the residue disappears. The sample was allowed to cool and weighed. The total ash was determined by the following equation. This is used to determine the quality and purity of the crude drug. Ash contains inorganic materials like Phosphates, carbonates, and silicates of sodium, potassium, magnesium, calcium etc. 2-3 g of powder drug was taken in crucible and kept it in the furnace at a temperature not exceeding 450ºC until it got free from carbon. The crucible was then cooled in a desiccator, and the ash weight was noted. Percentage of total ash was calculated with reference to the air dried sample of the crude drug.

%Total Ash Value = (W1-W2)/Wx100

Where,

W= Weight of the sample.

W1= Weight of ash + Crucible.

W2 = Weight of empty crucible.

Water soluble Ash value

Water soluble ash is the difference between total ash and water insoluble residue.The obtained ash was dissolved in 25 ml distilled water and kept aside for 5 min. The insoluble material was obtained by filtration using with Whatman paper grade (589/3) and then washed with hot water. The crucible was burnt for about 15-20 min maintained at 450°C; which indicates the absence of carbon. Insoluble matter was calculated by weighing the ash. Water soluble ash was calculated in term of percent by using the following formula.

% Water soluble Ash value = ((W1-W2)/W3)x100

Where,

W1= Weight of total ash

W2= Weight of water insoluble ash

W3= Weight of crude drug taken

Acid insoluble Ash value

Acid insoluble ash is a test used in order to determine the amount of inorganic residue present in a sample. This test is used for quality control in many industries, including the pharmaceutical and food industries. The acid insoluble ash test is also referred to as the silica test, or simply the ash test. The term acid insoluble ash (AIA) refers to the inorganic residues that remain after a sample is burned in order to determine its mineral content. Acid insoluble ash is used to measure the amount of minerals, such as silica, that are present in a sample. The procedure for measuring AIA is relatively simple: a known weight of the sample is burned in a furnace, and the resulting ash is weighed and then dissolved in hydrochloric acid. The final step is to measure the weight of the insoluble portion of the ash. Acid insoluble ash is a measure of the inorganic content of a sample. It is determined by heating the sample to 500 degrees Celsius and measuring the weight loss. Acid insoluble ash provides information on the purity of the sample and allows for comparison between different samples.

This represents the residue obtained after boiling the total ash with 25 ml (2M) Hydrochloric acid for 10 minutes. The matter insoluble was collected on Whatman filter paper grade (589/3) and clean with hot water burn in the crucible for about 15-20 min for the maintain the temperature not exceeding 450°C. Acid insoluble matter was calculated in terms of percentage by using the following formula.

The ash obtained from the total ash content, was boiled for 5 minutes with 25 ml of dilute hydrochloric acid, the insoluble matter obtained was collected on an ashless filter paper, which was washed with hot water and ignited the crucible in the flame. Then crucible is cooled, and weighed. The filter paper and residue was put together into the crucible, heated gently until vapours stops and heated further till carbon disappears. The crucible was cooled in a desiccator. The percentage of acid-insoluble ash was calculated using the formula

% Acid insoluble Ash value = (W1/W2)x100

Where,

W1= Weight of acid insoluble ash

W2= Weight of crude drug taken

Loss on drying (Moisture content)

5 g of the powdered drug was accurately weighed in pre-weighed Petri dish and dried under hot air oven for 120 minutes maintained at 1100C after that it was kept in desiccators at room temperature. The result was calculated by using the following formula.

% Loss on drying = (W1/W2)x100

Where,

W1= Loss in weight of sample

W2= Weight of sample

Water soluble extractive value

5 gm sample (crude drug) was macerated in 100 ml distilled water in a closed container for 6 hours with continuous stirring in orbital shaker assembly after that it was allowed to stand for 18 hr. Evaporate 25 ml filtrate till dryness and dried at 110°C. Weigh the sample without delay and calculate the extractive value.

Alcohol soluble extractive value

Alcohol soluble extractive value is the amount of alcohol required to completely extract a given volume of an herb or other material. This is typically expressed as a percentage of the total volume of the material being extracted. For example, if it takes 100 mL of alcohol to completely extract the active ingredients from 100 g of an herb, then that herb would have an alcohol soluble extractive value of 100%. This was estimated using the same procedure as in the case of water soluble and using alcohol in place of water.

Determination of pH Values

10 g powder was weighed, 100 ml distilled water was added to it so as to get 10% solution. It was then mixed continuously with the help of clean and dry glass rod for about 45 minutes till it became a homogenous mixture. It was then filtered through filter paper so as to remove the insoluble components. The pH meter was calibrated using standard buffer solution of pH 7 and pH 9 and then pH value was measured on pH meter.

Phytochemical of medicinal plant extracts

Preliminary phytochemical screening of the extracts has shown the presence of alkaloids, tannins, saponins, steroids and flavanoids done with standard methods.

Thin-layer chromatography

Thin-layer chromatography is performed on a sheet of an inert substrate such as glass, plastic, or aluminium foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide (alumina), or cellulose. This layer of adsorbent is known as the stationary phase. After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action. Because different analytes ascend the TLC plate at different rates, separation is achieved. It may be performed on the analytical scale as a means of monitoring the progress of a reaction, or on the preparative scale to purify small amounts of a compound. TLC is an analytical tool widely used because of its simplicity, relative low cost, high sensitivity, and speed of separation. TLC functions on the same principle as all chromatography: a compound will have different affinities for the mobile and stationary phases, and this affects the speed at which it migrates. The goal of TLC is to obtain well defined, well separated spots.

The particular single or mixture solvent for compound separation was used as the mobile phase and the absorbent materials are used as the stationary phase. The plant extracts were obtained by extraction techniques with different solvents from lower to higher polarity. The extracts were converted to crude extracts by distillation process with appropriate solvents.

The TLC plates were prepared by silica gel-G or Silica gel-GF (It shows Fluorescence under UV radiation). 30 gm of silica gel was weighed accurately and dispersed as a homogenous suspension with 60 ml distilled water for few minutes, then this homogenous suspension was evenly distributed over the TLC plate then plates were subjected to air dry. This TLC plates were dried into a hot air oven at 110°C for 30 minutes and then it stored in a moisture free atmosphere and used it whenever needed. Then the samples were prepared by diluting the crude extracts with a respective solvent or mobile phase. Then the samples were spotted on the TLC plate (1-10μl volumes) 2 cm above in the plates from the bottom with the help of a capillary tube. The movements of the active compounds were expressed by the retention factor (Rf). The Rf values were obtained from the phytochemicals provide the information about the polarity and separation of these phytochemical in the TLC separation process.

Different Rf values of the phytocompounds also give the idea about their polarity by the use of the various solvent systems. The TLC plates were slightly dried and spots were detected with the help of UV light at 254 nm (lower wave length) and 366 nm (higher wave length).

Rf = Distance travelled by solute / Distance travelled by solvent

RESULT AND DISCUSSION

Screening for phytochemicals is incredibly helpful in many different kinds of plant study. In the Physical-Chemical and Qualitative chapter, we conducted many qualitative tests for functional groups as well as tests of pH, extractive value, and ash value.

Physicochemical Standardization of Proposed Plant Drug

The following physicochemical properties were determined by normal technique using the powdered plant material of *Nigella sativa, Moringa olifera, and Curcuma longa.*

Identification, collection, and drying in the shade were done on the plants Nigella sativa, Moringa olifera, and Curcuma longa. The shade-dried leaves were ground into a powder that was somewhat gritty. This substance was ground up and utilised to determine different physicochemical characteristics. A number of physicochemical characteristics were calculated, including swelling index, ash content, acid insoluble ash, and water soluble ash extractive value. *Nigella sativa, Moringa olifera, and Curcuma longa* medication extracts were reported to have ash content values of 7.20 % w/w, 7.13% w/w, 7.06% w/w, and 7.05% w/w, respectively. While *Nigella sativa, Moringa olifera, and Curcuma longa* medicine extract were found to have water soluble ash contents of 3.92%, 3.94%, 4.23%, and 4.61%, respectively. An analysis of the acid insoluble ash revealed that Nigella sativa, *Moringa olifera*, and *Curcuma longa*, respectively, were of drug ext. The table below shows the values that were obtained:

Table 5.1. Standardization parameters of *Nigella sativa, Moringa olifera* and *Curcuma longa*

S.No	Parameters % w/w	Nigella sativa	Moringa oleifera	Curcuma Longa
1.	Ash value	7.02	7.13	7.06
2.	Swelling index	0.14	0.13	0.16
3.	Water soluble ash	3.92	3.94	4.23
4.	Acid insoluble ash	1.83	1.84	1.88

Extraction of Selected Plant Drug

The active ingredients were extracted from the relatively coarse powder of the leaves of *Nigella sativa*, *Moringa olifera*, and *Curcuma longa* using water as the solvent. The obtained aqueous extract of *Moringa olifera* and Nigella sativa leaf was dried and weighed. According to accepted procedures, the % yield of plant-based drugs was determined. The measured plant medicine extract was kept in desiccators for further use. The yield of the aqueous extract with semisolid masses of brownish, greenish, and bright yellow colours, respectively, was determined to be 6.37, 7.83, and 4.23% w/w of the crude drug. Results obtained were listed in a table.

Table 5.2 Extractive values obtained from *Nigella sativa*, *Moringa olifera* and *Curcuma longa*

S.No	Solvent	Color of extract	% yield
1.	Nigella sativa	Brownish	6.37
2.	Moringa olifera	Greenish	7.83
3.	Curcuma longa	Fluorescent yellow	4.23

Phytochemical Screening of Extract

After extraction, several extracts were tested using phytochemicals to identify the different classes of medicinal components contained in *Nigella sativa*, *Moringa olifera*, and *Curcuma longa*.

Table 5.3 Identification test for alkaloids in Nigella sativa

S.NO	TEST	INFERENCE
1	Dragendorff's test: With Dragendorff's reagent (solution of potassium bismuth iodide)	**Positive**
2	Mayer's test: With Mayer's reagent (potassium mercuric iodide solution)	**Positive**
3	Hager's test: With Hager's reagent (saturated picric acid solution)	**Positive**
4	Wagner's test: With Wagner's reagent (solution of iodine in potassium iodide).	**Positive**

Table 5.4 Identification test for glycosides in *Nigella sativa*

S.NO	TEST	INFERENCE
1	**Legal's test:** To the methanolic extracts, added pyridine and sodium nitroprusside	**positive**
2	**Borntrager's test:** Different extracts were boiled with dilute sulphuric acid and filtered. To the cold filtrates equal volumes of chloroform were added. After thorough shaking the organic solvent layers were separated and ammonia solution was added.) **Modified Borntager test:** 5ml extract+ 5ml 5% $FeCl_3$ + Heat (5mins). Cool it and add benzene. Separate organic layer. Dil ammonia	**Positive** **Positive**
3	**Sodium picrate test:** Soaked filter paper strips first in 10% picric acid and then in 10% sodium carbonate and dried + Extract	**Negative**

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Table 5.5 Tests for flavonoids in *Nigella sativa*

S.NO	TEST	INFERENCE
1	**Shinoda test:** extract+5ml 95 % alcohol, few drops of conc. HCL + 0.5g Mg	**Negative**
2	**Sulphuric acid test:** extract + 80% sulphuric acid	**Negative**

Table 5.6 Identification test for alkaloids in *Moringa olifera*

S.NO	TEST	INFERENCE
1	Dragendorff's test: With Dragendorff's reagent (solution of potassium bismuth iodide)	**Positive**
2	Mayer's test: With Mayer's reagent (potassium mercuric iodide solution)	**Positive**
3	Hager's test: With Hager's reagent (saturated picric acid solution)	**Positive**
4	Wagner's test: With Wagner's reagent (solution of iodine in potassium iodide).	**Positive**

Table 5.7 Identification test for glycosides in *Moringa olifera*

S.NO	TEST	INFERENCE
1	**Legal's test:** To the methanolic extracts, added pyridine and sodium nitroprusside	**positive**
2	**Borntrager's test:** Different extracts were boiled with dilute sulphuric acid and filtered. To the cold filtrates equal volumes of chloroform were added. After thorough shaking the organic solvent layers were separated and ammonia solution was added.) **Modified Borntager test:** 5ml extract+ 5ml 5% $FeCl_3$ + Heat (5mins). Cool it and add benzene. Separate organic layer. Dil ammonia	**Positive** **Negative**
3	**Sodium picrate test:** Soaked filter paper strips first in 10% picric acid and then in 10% sodium carbonate and dried + Extract	**positive**

Table 5.8 Tests for flavonoids in *Moringa olifera*

S.NO	TEST	INFERENCE
1	**Shinoda test:** extract+5ml 95 % alcohol, few drops of conc. HCL + 0.5g Mg	**Positive**
2	**Sulphuric acid test:** extract + 80% sulphuric acid	**Positive**

Table 5.9 Identification test for alkaloids in *Curcuma longa*

S.NO	TEST	INFERENCE
1	Dragendorff's test: With Dragendorff's reagent (solution of potassium bismuth iodide)	**Positive**
2	Mayer's test: With Mayer's reagent (potassium mercuric iodide solution)	**Positive**
3	Hager's test: With Hager's reagent (saturated picric acid solution)	**Positive**
4	Wagner's test: With Wagner's reagent (solution of iodine in potassium iodide).	**Positive**

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Table 5.10 Identification test for glycosides in *Curcuma longa*

S.NO	TEST	INFERENCE
1	**Legal's test:** To the methanolic extracts, added pyridine and sodium nitroprusside	**Positive**
2	**Borntrager's test:** Different extracts were boiled with dilute sulphuric acid and filtered. To the cold filtrates equal volumes of chloroform were added. After thorough shaking the organic solvent layers were separated and ammonia solution was added.) **Modified Borntager test:** 5ml extract+ 5ml 5% $FeCl_3$ + Heat (5mins). Cool it and add benzene. Separate organic layer. Dil ammonia	**Negative** **Negative**
3	**Sodium picrate test:** Soaked filter paper strips first in 10% picric acid and then in 10% sodium carbonate and dried + Extract	**positive**

Table 5.11 Tests for flavonoids in Curcuma longa

S.NO	TEST	INFERENCE
1	**Shinoda test:** extract+5ml 95 % alcohol, few drops of conc. HCL + 0.5g Mg	**Positive**
2	**Sulphuric acid test:** extract + 80% sulphuric acid	**Positive**

CONCLUSION

A number of physicochemical characteristics were calculated, including swelling index, ash content, acid insoluble ash, and water soluble ash extractive value. *Nigella sativa, Moringa olifera, and Curcuma longa* medication extracts were reported to have ash content values of 7.20 % w/w, 7.13% w/w, 7.06% w/w, and 7.05% w/w, respectively. While *Nigella sativa, Moringa olifera, and Curcuma longa* medicine extract were found to have water soluble ash contents of 3.92%, 3.94%, 4.23%, and 4.61%, respectively. An analysis of the acid insoluble ash revealed that *Nigella sativa, Moringa olifera*, and Curcuma longa, respectively, were of drug ext. The yield of the aqueous extract with semisolid masses of brownish, greenish, and bright yellow colours, respectively, was determined to be 6.37, 7.83, and 4.23% w/w of the crude drug. After extraction, several extracts were tested using phytochemicals to identify the different classes of medicinal components contained in *Nigella sativa, Moringa* olifera, and Curcuma longa and are listed in the table above.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest.

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