International Journal of Technology Research and Management
ISSN (Online): 2348-9006
Vol 8 Issue 6 June 2021

Pharmacognostical, Phytochemical Studies and Pharmacological Evaluation of Indian Kamala

Nitin Kumar[1], Shipra[2], Dr. R. K. Singhal[3], Vidhan Singhal[4], Narottam Singh[5], Ajay Kumar[6]
Keshav Pharmacy College, Muzaffarnagar,(AKTU)(BTEUP)Lucknow (U.P)(India) 251310
Oxford College of Pharmacy, Ghaziabad,(AKTU)(BTEUP)Lucknow (U.P)(India) 201001
nitinkr862@gmail.com[1], siyabaish@gmail.com[2], keshavpharmacymzn@gmail.com[3]

Abstract: *The present study was aimed at Pharmacognostical, Phytochemical and Pharmacological evaluation of the plant (Indian Kamala) family- Euphorbiaceae. Pharmacognostical investigation were carried out by performing organoleptic, microscopical and physicochemical evaluation i.e. ash values, extractive values, moisture content, swelling index, foaming index and foreign matter. The obtained results showed that the moisture content was found to be 1.74%. Similarly swelling index was recorded to be (0.8cm). Foaming index (less than 100). Phytochemical investigation included successive soxhlet extraction, the obtained extractive values were 4.9%, 5.45%, 9.77%, 8.75%, and 4.4% for petroleum ether, chloroform, ethyl acetate, ethanol and distilled water respectively. The preliminary qualitative phytochemical screening revealed the presence of carbohydrates, phenolic compounds, alkaloids, glycosides, flavonoids, steroids, fixed oils and fats. Pharmacological investigation included the Anthelmetic activity by using the (Indian Kamala) plant ethyl acetate extract. The 120mg/kg dose to be found positively in Pharmacological response.*

1. INTRODUCTION

The plants employed for medicinal purpose are considered to include all plant material such as flower, fruit, root, foliage and seed which may be useful as such or in the form of extracts and chemical compounds isolated from them to produce drugs for human and veterinary medicine. These plants are closely related to those that produce stimulants, condiments, spices, essential oils, and such other higher forms of plants life that produce specific influence on cell metabolism. Among the kingdom of crude drugs (plants, animals and minerals), medicinal plants were first to be used by men. Medicinal plants account for 20% of all medical prescriptions in industrialized countries and for 80% in developing countries. The World Health Organization (WHO) has compiled a list of over 20,000 common medicinal plants used in different parts of the globe and many of them are known for their efficacy against different human ailments. India has been endowed with a very rich flora due to the extreme variations in geographical and climatic conditions. These plants have been used since ancient times for the treatment of human diseases. The traditional system of medicines (Ayurveda, Siddha and Unani-Tibb) together with folklore medicine still continues to serve, in spite of the advent of modern medicine, to large portions of the population, particularly in the rural areas. India is one of the world's twelve leading biodiversity centres with the presence of over 45,000 different plant species, of which about 15,000-20,000 plants have got medicinal values. However, only about 7,000-7,500 is used for their medicinal values by traditional communities. The medicinal potential of plant drugs is well recognized now, as for instance, the consumption of medicinal plants has doubled in last ten years in Western Europe. It has been estimated that up to 50% of the prescriptions presently dispensed in USA may contain one or more natural product drugs. It seems certain that the continued scientific study of medicinal plant will afford a plethora of novel, structurally diverse bioactive compounds. The WHO has emphasized the utilization of indigenous system of medicine based on the ideally available raw materials i.e. medicinal plant [1].

The current success of taxol, in particular, should encourage additional industrial, academic and government laboratories to engage in research on the discovery and development of plant-derived drugs. Multidisciplinary research on plants should lead to many new drug candidates as well as to prototype biologically active molecules and biological tools. [2]

The World Health Organization (WHO) defines traditional medicine as the "Diverse health practices, approaches, knowledge and beliefs incorporating plant, animal and/or mineral based medicines, spiritual therapies, manual techniques and exercises applied singularly or in combination to maintain well-being, as well as to treat, diagnose, or prevent illness". It is clear, however, that there is a need to validate the information through an organised infrastructure for it to be used as an effective therapeutic means, either in conjunction with existing therapies, or as a tool in novel drug discovery. Traditional medicine utilises biological resources and the indigenous knowledge of traditional plant groups, the latter being conveyed verbally from generation to generation. This is closely linked to the conservation of biodiversity and the related intellectual property rights of indigenous people [3].

1. Standardization

Standardization refers to "Adjusting the herbal drug preparation to a defined content of a constituents or a group of substances with known therapeutic activity". Medicinal plants are widely used in the formulation of herbal based healthcare products. Although herbal remedies are often perceived as being natural and safe, but they are not free from adverse effects. Adverse effects of herbal medicine may be due to factors such as adulteration, substitution, contamination, misidentification, incorrect preparation and/or doses, lack of standardization and inappropriate labelling. The major criticism being faced by traditional system of medicine is inadequate scientific validation and standards of plant material employed by the manufacturer in the herbal formulations. Thus there is a strong need in promoting standardization of quality parameters of important medicinal herbs. WHO emphasized the need to ensure quality control of medicinal plant products by using modern techniques and applying suitable standards. Several pharmacopoeias including United States Pharmacopoeia, Indian Pharmacopoeia, British Pharmacopoeia and Japanese Pharmacopoeia covers monographs and quality control tests for few of the medicinal plants used in the respective country [4]

2. Pharmacological Screening

When the estimation of potency of crude drug or its preparation is done by means of its effect on living organisms like bacteria, fungal growth or animal tissue or entire animal, it is known as bioassay. This method is generally used for conformity of therapeutic activity of raw material and finished product and also, when standardization is not adequately done by chemical or physical means. Biological assay methods are mainly of three types -

i. Toxic
ii. Symptomatic
iii. Tissue methods

In toxic and symptomatic techniques, the animals are used, whereas in tissue method, the effect of a drug is observed on isolated organs or tissues. In standardization or evaluation of herbal drugs, assessment of biological efficacy is found to be mostly used method. In all these methods, requirements are suitable animal models for testing and control, methodology for experiment and assessment for results. Protocols followed for assessment of some types of biological activities have been revised by Farnsworth, 1996. Well established bioassays are available for number of pharmacological activities such as: Hepatoprotective activity, Neurpharmacological activity, Anti-ulcer activity, Antidiabetic activity, Antiinflammatory activity, Antimicrobial activity, Antioxidant activity, Cytotoxicity etc. [5].

2. ANTHELMINTICS

Anthelmintics are drugs that are used to treat infections with parasitic worms. This includes both flat worms, e.g., flukes and tapeworms and round worms, i.e., nematodes. They are of huge importance for human tropical medicine and for veterinary medicine. The World Health Organization estimates that a staggering 2 billion people harbour parasitic worm infections (http://www.who.int/wormcontrol/statistics/). Parasitic worms also infect livestock and crops, affecting food production with a resultant economic impact. Also of importance is the infection of domestic pets. Indeed, the companion animal market is a major economic consideration for animal health companies undertaking drug discovery programmes.[6]

Despite the prevalence of parasitic worms, anthelmintic drug discovery is the poor relation of the pharmaceutical industry. The simple reason is that the nations which suffer most from these tropical diseases have little money to invest in drug discovery or therapy. It comes as no surprise therefore that the drugs available for human treatment were first developed as veterinary medicines. There is thus a pitifully small repertoire of chemotherapeutic agents

available for treatment (see Table 1). In some respects, this situation has been exacerbated by the remarkable success of ivermectin over the last twenty years which has decreased motivation for anthelmintic drug discovery programmes. This prompts concern, as anthelmintic resistance has been widely reported in livestock and it may also only be a matter of time before this phenomenon occurs in parasites of humans[7]

Table 1: Key drugs registered for the treatment of parasitic worms in humans

Schistosomiasis (blood fluke)	**Intestinal round worms**
Antimonials	Piperazine
Metrifonate	Benzimidazoles
Oxamnaquine	Morantel
Praziquantel	Pyrantel
	Levamisole
Cestodiasis (tape worm)	Avermectins and milbemycins
Niclosamide	Closantel (and halogenated salicylamides)
Benzimidazoles	Emodepside
Praziquantel	
Fasciolasis (liver fluke)	**Filariasis (tissue round worms)**
Praziquantel	Diethylcarbmazine
Closantel	Suramin
(and halogenated salicylamides)	Ivermectin

Broad spectrum anthelmintics are effective against parasitic flat worms and nematodes. However, the majority of drugs are more limited in their action, e.g., praziquantel, a drug used in the treatment of schistosomiasis and thought to act by disrupting calcium homeostasis (Greenberg, 2005), has no activity against nematodes (see Table 1). For the purpose of this review we will focus on drugs used in human and veterinary medicine to treat parasitic nematode infection.[8]

3. METHODOLOGY

1. Aims and Objectives

It comprised of consecutive three steps:

Part A: Pharmacognostical Studies
Part B: Phytochemical Studies
Part C: Pharmacological Studies

Part A: Pharmacognostical Investigation

It included collection, identification and authentication of plant material, drying and size reduction, organoleptic evaluation, microscopic evalution (transerverse section of leaf, stem and root), powder microscopy and determination of leaf constant.

Physico-chemical investigation included determination of foreign organic matter, ash value (total ash, acid insoluble ash and water soluble ash), extractive value, moisture content (loss on drying), swelling index and foaming index. [37]

Part B: Phytochemical investigations

It included extraction (successive soxhlet extraction with increasing polarity of various solvents- petroleum ether, chloroform, ethyl acetate, ethanol and water), phytochemical screening (chemical tests of various extracts) and fluorescence analysis.

Part C: Pharmacological Study

Evaluation of *invitro* anthelmintic activity

All the experiments were carried out in Indian adult earthworms (*Indian Kamala)* due to its anatomical resemblance with the intestinal roundworm parasites of human beings. They were collected from moist soil and washed with water to remove all fecal matters. [38]

Preparation of extracts

The plant leaf powder was subjected to hot water maceration to obtain aqueous extracts. The dry powder is extracted with benzene and methanol using maceration process for 48 hours. The powdered (*Indian Kamala)* was extracted exhaustively with increasing polarity solvents (methanol, benzene and water) for 72 hours followed 48 hours and 24 hours. The solvents were pooled, distilled under vaccum and dried under vaccum dessicator. Different concentrations (25, 50, 100 and 200mg) of each extract solution were prepared by diluting the stock solution, in propylene glycol, using normal saline. [39]

Part A: Pharmacognostical Investigation

Organoleptic evaluation

It referred to the evaluation of a drug by colour, odour, size, shape, taste and special features such as: touch, fracture, texture etc. which was evaluated with the help of sense organs [40].

Microscopic evaluation

The histological studies of the (*Indian Kamala)* plant (aerial part) included transverse section examination, powder microscopy and determination of leaf constant etc [78]

- Transverse section examination of stem, fruit and flower

- Powder microscopy

PHYSICO-CHEMICAL INVESTIGATION

Such an investigation is carried out with reference of following parameters-

- **Extractive value**

This determines the amount of constituents extracted with solvents from a given amount of plant material.

- **Foreign organic matter**

It was examined by taking 100-500 gm of the drug sample weighed and spread on a white tile uniformly without overlapping. The foreign organic matter was separated manually and examined under day light with eye. The suspected particles were transferred into a petridish. After complete separation, weight of the foreign matter was taken and % w/w was determined as mentioned in table.[41]

- **Ash value**

Ash value is an important parameter for the purpose of evaluation of crude drugs. The ash of any organic material is composed of their non-volatile inorganic components. The ash remaining following ignition of medicinal plant materials is determined by three different methods, which measure total ash, acid-insoluble ash and water-soluble ash [42].

- **Moisture content(loss on drying)**

This parameter determines the amount of moisture as well as volatile components present in a particular sample [43].

- **Swelling index**

Many plant materials are of specific therapeutic or pharmaceutical utility because of their swelling properties, especially gums and those containing an appreciable amount of mucilage, pectin or hemi cellulose. The swelling index is the volume in ml taken up by swelling of 1gm of plant material under specified conditions [44].

- **Foaming index**

Many medicinal plant materials contain saponins that can cause persistent foam when an aqueous decoction is shaken. The foaming ability of an aqueous decoction of plant material and their extract is measured in terms of foaming index [45].

PART B: PHYTOCHEMICAL STUDIES

Successive extractive value

The powdered material of the drug (10gm) was packed in a soxhlet apparatus and was subjected to successive extraction with different solvent like petroleum ether, chloroform, ethyl acetate, ethanol and water. The extracts were evaporated to dryness and their extractive values were calculated [46].

Chemical Tests for Detection of Organic Constituents

The preliminary phytochemical screening is carried out using different plant extracts for their content of different classes of compounds. The extract obtained is subjected to qualitative Chemical tests for identification of various plant constituents present in the crude drug. The extract is subjected to preliminary phytochemical investigation for detection of following constituents [47].

1. Alkaloids
2. Carbohydrates
3. Glycosides
4. Phenolic compounds
5. Flavonoids
6. Protein and amino acids
7. Saponins
8. Mucilage
9. Resins
10. Lipids/Fats

Fluorescence Analysis

The plant (*Indian Kamala)* powder was treated with different reagents and examined under UV light (254 and 366 nm) [48].

PART C: PHARMACOLOGICAL STUDIES

Evaluation of *invitro* anthelmintic activity

All the experiments were carried out in Indian adult earthworms (*Indian Kamala)* due to its anatomical resemblance with the intestinal roundworm parasites of human beings. They were collected from moist soil and washed with water to remove all fecal matters. [38]

Preparation of extracts

The plant leaf powder was subjected to hot water maceration to obtain aqueous extracts. The dry powder is extracted with benzene and methanol using maceration process for 48 hours. The powdered (*Indian Kamala)* was extracted exhaustively with increasing polarity solvents (methanol, benzene and water) for 72 hours followed 48 hours and 24 hours. The solvents were pooled, distilled under vaccum and dried under vaccum dessicator. Different concentrations (25, 50, 100 and 200mg) of each extract solution were prepared by diluting the stock solution, in propylene glycol, using normal saline.[39]

A: PHARMACOGNOSTICAL INVESTIGATION

It includes collection, identification and authentication of plant material, drying and size reduction, organoleptic evaluation, microscopic evaluation (transerverse section of leaf, stem and root), powder microscopy, determination of stomatal number, stomatal index, palisade ratio, vein-islet number and vein termination number. Physico-chemical

investigation includes determination of foreign organic matter, ash value (total ash, acid insoluble ash and water soluble ash), extractive value, moisture content (loss on drying), swelling index and foaming index.

Collection of plant material

The plant was collected from the Drug market, Muzaffarnagar, UP.

Drying and size reduction

The authenticated plant was dried under shade and then coarsely powdered with the help of mechanical grinder. The coarse powder was stored in an airtight container for pharmacognostical studies and successive extraction.

Organoleptic Evaluation

It was carried out by evaluating the colour, odour, size, shape, taste and special features such as: touch, fracture,texture etc.

Microscopic Evaluation

Transverse section examination of stem and flower

It was carried out by using the fresh (*Indian Kamala)* plant parts for section cutting. Stems and leaf were soaked in chloral hydrate for few minutes in order to make them soft and then the cross sections were prepared by taking free hand section. The thin section of the stem, leaf and flower were selected and stained with chemical dye (colorant), safranin, iodine solution , sudan red III and mounted with the help of glycerin. The finally prepared slides were then captured through compound microscope and labeled .The labeled characters was as shown in figure.

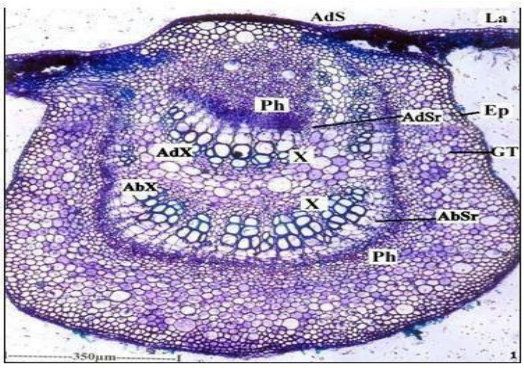

Figure 1 : Transverse Section of leaf through Midrib (10X)
(AdS – Adaxial side, AbSr – Abaxial strand, AdSr - Adaxial strand, AbX – Abaxial xylem, AdX – Adaxial xylem, Ep – Epidermis, GT – Ground Tissue, La – Lamina, Ph Phloem, X – Xylem)

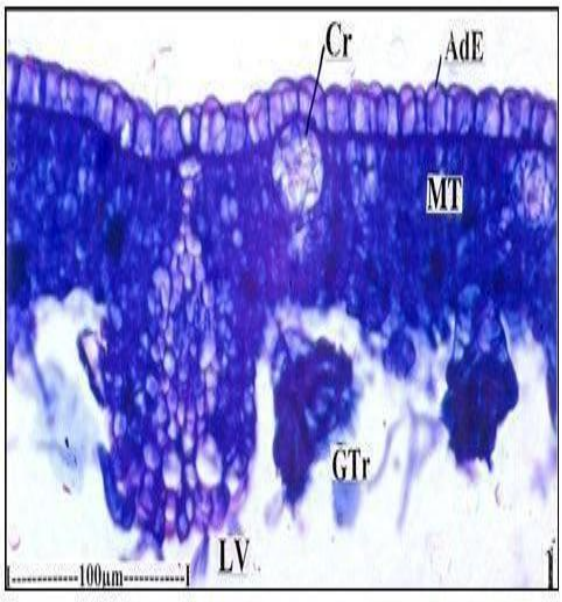

Figure 2 : Transverse section of lamina showing crystals, glandular and nonglandulatrichomes.(40X)
(AdE – Adaxial epidermis; Cr – Crystal; GTr. - glandular trichomes; LV – Lateral vein; MT – Mesophyll tissue)

Powder microscopy

It was done by using the fine powder of the plant parts of (*Indian Kamala)* The powder was treated with chloral hydrate for few minutes. Further this powder drug was transferred on a microscopy slide with a drop of glycerin to prevent drying, a cover- slip was then placed on the powdered drug and finally the slide was observed under microscope. Different cells, tissues and their arrangement was observed and identified from the microscopic slide and mentioned in table.

Stomatal Number

It was done by clearing a piece of the stem placing in a test tube containing about 5 ml of chloral hydrate solution and heated on a boiling water-bath for about 15 minutes. Upper and lower epidermises were peeled out using a forecep. A fragment was transferred to a microscopic slide and prepared to mount a small drop of glycerol-ethanol solution was placed on one slides of the cover-glass. The slide was examined with a 40x objective and a 6x eye piece, to which a microscopical drawing paper a square of 1mm with the help of stage micrometer was drawn.

The epidermal cells and stomata were traced. The number of stomata present in the area of 1 sq. mm was counted. The cell was included if at least half of its area lied within the

square. The average number of stomata per square millimetre for each surface of the stem was calculated and mentioned in table.

Stomatal Index

It was done by clearing a piece of the stem placing in a test tube containing about 5 ml of chloral hydrate solution and heated on a boiling water-bath for about 15 minutes and mounted. The lower surface examined by means of a microscope with a 4 mm objective and an containing a 5 mm square micrometer disc. Counts made of the numbers of epidermal cells and of stomata (the two cells and ostiole considered as one unit) within the square a cell being counted if at least half of its area lies within the grid. A successive adjacent field was examined until about 400 cells had been counted and the stomatal index value calculated from these figures. The stomatal index determined for both stem surfaces and mentioned in table.

The percentage proportion of the ultimate division of the epidermis of a leaf which have been converted into stomata is termed the stomatal index.

Stomatal index =SE+S×100

Where S = number of stomata per unit area and *E* = number of ordinary epidermal cells in the same unit area.

Vein islet number

It was done by clearing a piece of the stem placing in a test tube containing about 5 ml of chloral hydrate solution and heated on a boiling water-bath for about 15 minutes and mounted. A camera lucida was set up and by means of a stage micrometer the paper is divided into squares of 1sq.mm using a 16 mm objective. The stage micrometer was then replaced by the cleared preparation and the veins were traced in four contiguous squares. Number of vein islet number were counted and mentioned in table.

Vein termination number

It was carried out by clearing a piece of the stem placing in a test tube containing about 5 ml of chloral hydrate solution and heated on a boiling water-bath for about 15 minutes and mounted. A camera lucida was set up and by means of a stage micrometer the paper is divided into squares of 1sq.mm using a 16 mm. The veins were traced off which were included within the square completing the outline of those islets which overlap to adjacent slides of the square. The number of vein termination were counted in the sq. mm where the islets inserted by the sides of square, including those on two adjacent sides and excluding those islets on the other sides. The average number of vein termination from the four adjoining square was counted to get value for one square millimeter. Number of vein termination number were counted and mentioned in table.

PHYSICO-CHEMICAL INVESTIGATION

It was investigate with reference to foreign organic matter, ash value (Total ash, acid insoluble ash and water soluble ash), extractive value, moisture content (loss on drying), swelling index, foaming index.

Foreign organic matter

It was examined by taking 100-500 gm of the drug sample weighed and spread on a white tile uniformly without overlapping. The foreign organic matter was separated manually and examined under day light with eye. The suspected particles were transferred into a petridish. After complete separation, weight of the foreign matter was taken and % w/w was determined as mentioned in table.

Ash value

Total ash

It was measured by accurately weighing (about 2-3 g) of air dried crude drug placed in the silica dish and was incinerated at a temperature not exceeding 450 °C until free from carbon, cooled and weighed to get the total ash content as mentioned in table.

% Total Ash value =weight of total ashweight of crude drug taken×100

Acid insoluble ash

It was done by boiling ash with 25ml of hydrochloric acid for 5 minutes. The insoluble matter was collected on ash less filter paper, washed with hot water and ignited at a temperature not exceeding 450^{o}c to a constant weighas mentioned in table.

% Acid insoluble ash value =weight of acid insoluble ashweight of crude drug taken×100

Water soluble ash

It was obtained by dissolving ash in distilled water and the insoluble part collected on an ash less filter paper and was ignited at 450^{o}C to a constant weight. By subtracting the weight of insoluble part from that of ash, the weight of the soluble part of ash was obtainedas mentioned in table.

% Water soluble ash value =weight of total ash-weight of water insoluble ashweight of crude drug taken×100

Alcohol soluble extractive

It was done by taking 5 g of coarsely powdered and macerated drug with 100ml ethanol in a closed flask for 24 hours. The flask was shaken frequently during 6 hours and allowed to stand for 18 hours. After that the extract was filtered rapidly and evaporated upto dryness in china dish. The percentage yield of ethanol-soluble extractive with

reference to the air dried drug was finally calculated as shown in table.

% yield=Final weight of the extract Amount of the crude drug taken×100

Moisture content (Loss on drying)

It was determined by taking powdered drug sample (10gm) placed on a tarred evaporating dish and dried at 105°C for 6 hours and weighed. The drying was continued until two successive reading matches each otheras mentioned in table .

% Loss on drying =Loss in weight of the sampleweight of the sample×100

Swelling index

It was determined by taking accurately weighed powder drug into 25ml glass stopped measuring cylinder. 25ml of water was added and the mixture was shaken thoroughly every 10 minutes for 1 hour. It was allowed to stand for 3 hours at room temperature. The mean value of the individual determinations was calculated related to 1gm of plant materialas mentioned in table.

Foaming index

It was observed by taking 1gm powdered drug reduced to a coarse powder (sieve size no. 1250), weighed accurately and transferred to 500ml conical flask containing 100ml of boiling water.Maintained at moderate boiling for 30 minutes. Cooled and filtered into 100ml volumetric flask. The detection was poured into 10ml stopped test tube (height 16cm, diameter 16mm) in successive portions of 1ml, 2ml, 3ml etc. up to 10ml and adjusted the volume of liquid in each tube with water to 10ml.Stoppered the tube and was shaken them in a lengthwise motion for 15 sec, two shakes per second. Allowed to stand for 15min and the height of foam were measured.

The results were assessed as follows:

a) If the height of foam in every tube was less than 1cm the foaming index was less than 100.

b) If the height of foam in any tube is 1cm, the volume of plant material decoction in the tube was used to determine the index. If this tube was the first or second tube in a series, an intermediate dilution was prepared in a similar manner to obtain a more precise result.

c) If the height of the foam was more than 1cm in every tube, the foaming index was over 1000.In this case the determinations were repeated using a new series of decoction of detection in order to obtain a result.

The foaming index using the following formula:

$$\frac{1000}{a}$$

Where a = the volume in ml of decoction used for preparing the dilution in the tube where foaming to a height of 1cm was observed and mentioned in table.

B: PHYTOCHEMICAL INVESTIGATIONS

It included extraction (successive soxhlet extraction with increasing polarity of various solvents- petroleum ether, chloroform and ethanol), phytochemical screening (chemical tests of various extracts) and fluorescence analysis.

Successive Extraction

On the basis of polarity different solvents like petroleum ether, benzene, chloroform, ethyl acetate, ethanol and distil water are chosen for successive soxhlet extraction. Petroleum ether, a non-polar solvent used for separation of chlorophyll and fats from the plant material. Benzene, a lower medium polar solvent, Chloroform and ethyl acetate are medium polar solvent, methanol a more polar solvent, water is a high polar solvent. The aim of choosing so many polar solvents is to separate the different secondary plant metabolites of different polarities.

DISTILLATION AND CONCENTRATION:

The extracts were concentrated by vacuum distillation and pure solvent was collected. Finally they were made semisolid by heating on water bath and stored in desiccators.

EXTRACTIVE YIELD OF DIFFERENT EXTRACTS:

The % yields of different extracts were calculated as follows.

$$\% \text{ Yield of a extract} = \frac{\text{Weight of the semisolid extract}}{\text{Weight of powder taken for extraction}} \times 100$$

350gm of powdered thalamus was taken for the extraction with petroleum ether by reflux condensation process. The extract was concentrated and the % yield was determined and reported in the table.

4. QUALITATIVE PHYTOCHEMICAL ANALYSIS

The phytochemical Analysis is done to identify the secondary Phytoconstituents (plant metabolites) present in the different extracts.

Different extracts obtained from the above extraction processes and form the reflux condensation process were analyzed for different secondary Phytoconstituents present in these by the method of qualitative phytochemical analysis. The following chemical tests were carried out and the results were tabulated in table.

TEST FOR ALKALOIDS

Wagner's Reagents: -

With alkaloid it shows reddish-brown precipitate. It is prepared by dissolving 1.27 gm of iodine and 2gm of potassium iodide in 5 ml of water and the final volume is making up to 200 ml.

Mayer's Reagents: -

It is another method of detecting alkaloids. With alkaloids, it shows white to buff precipitate. To prepare the reagent, 1.36 gm of mercuric chloride is dissolved in distilled water. In another part dissolve 5 gm of potassium iodide in 60ml of distilled water. Then both the parts were mixed and volume was adjusted to 200ml.

Dragendroff's Reagents: -

With alkaloids, this reagent gives orange-brown colour precipitate. To prepare this reagent, 14gm of sodium iodide was boiled with 5.2 gm of bismuth carbonate in 50 ml of glacial acid for a few minutes. Then it was allowed to stand for over-night and the precipitate of sodium acetate was filtered out. To 40ml of filtrate 160ml of acetate and 1 ml of water was added. The stock solution was stored in amber-colour bottle. During experiment, to 10ml of this stock solution, 20ml of acetic acid was added and the final volume was made up to 100 ml with water.

Hager's Reagents: -

This reagent shows characteristic crystalline precipitate with many alkaloids. In this case a saturated aqueous picric acid was used for detection of alkaloids.

TEST FOR CARBOHYDRATES

Benedict 's test

In this method of test for monosaccarides, 5ml of the Benedict's reagent and 3ml of the test solution when boiled on a water-bath and brick-red ppt. Appears at the bottom of the test tube conforms the presence of the compounds.

Fehling's Test

In this method to about 2ml of Fehling's solution A and 2ml of Fehling's solution B, 2ml of the extract was boiled. The presence of reducing sugar is conformed if yellow or brick red ppt. appears.[57]

Molisch's Test

When the aqueous or alcoholic solution of the extract and 10% alcoholic solution of α-napthol were shaken and conc. Sulphuric acid was added along the side of the test tube, a violet ring at the junction of two liquids conforms presence of carbohydrates.

TEST FOR GLYCOSIDES

Test for cardiac glycosides

* **Keller- Killiani Test**

To an extract of the drug in glacial acetic acid, few drops of ferric chloride and conc. H_2SO_4 acid are added. A reddish brown colour is formed at the junction of two layers and upper layer turns bluish green.

* **Legal Test**

To a solution of glycoside in pyridine, sodium nitroprusside solution and sodium hydroxide solution were added. A pink to red colour will conform the presence of glycosides.

TEST FOR ANTHRAQUINONE GLYCOSIDES

* **Brontrager's Test**

To perform this test, 0.1gm of the powdered drug was boiled with 5ml of 10% sulphuric acid for 2 min. It was filtered while hot, then cooled and the filtrate was shaken with equal volume of benzene. The benzene layer was allowed to separate completely from the lower layer, which was pipetted out and transferred to a clean test tube. Then half of its volume of aqueous ammonia (10%) was added and shaken gently and the layers were allowed to separate. The lower ammonia layer will so show red pink colour due to presence of free Anthraquinones.

Modified Borntrager's Test

The C-Glycosides of Anthraquinones requires more drastic conditions for hydrolysis and thus a modification of the above test is to use ferric chloride and hydrochloric acid to affect oxidative hydrolysis.

When 0.1 gm of the drug, 5ml of dilute. HCL and 5 ml of 5% solution of ferric chloride were added and boiled for few minutes and the subsequently cooled and filtered part is shaken with benzene; the separated benzene layer and then add equal volume of dilute solution of ammonia which shows pink colour.

TEST FOR GUMS AND MUCILAGES

* **Molisch's Test**

When aqueous or alcoholic solution of the extract and 10% alcoholic solution of α –napthol were shaken and conc. H_2SO_4 added to the side of the test tube, appearance of violet ring at the junction of two liquids indicates the presence of carbohydrates, gums and mucilage's.

* **Precipitated with 95% Alcohol**

When 95% of alcohol added to the extract, gums and precipitate outs being insoluble in alcohol.

TEST FOR PROTEINS AND AMINO ACIDS

* **Biuret Test**

When 2ml of the extract, 2ml 10% NaOH solution and 2-3 drops of 1% $CuSO_4$ solution were mixed, the appearance of violet or purple colourconforms the presence of proteins.

* **Ninhydrin Test**

When 0.5ml of ninhydrin solution if added to 2 ml of the extract and boiled for 2 minutes and then cooled, appearance of blue colour conforms the presence of proteins.

* **Xanthoproteic Test**

When 2ml of extract and 1 ml of conc. HNO_3 were boiled and cooled and subsequently 40% NaOH solution added drop by drop to it, appearance of colour solution indicates the presence of proteins.[60]

* **Millon's Test**

When 2 ml of extract and 2ml of millon's reagents are boiled and subsequently cooled and then few drops of $NaNO_2$ were added to it, appearance of red precipitate and red colour solution indicates presence of proteins.

TEST FOR TANNINS AND PHENOLIC COMPOUNDS

* **With Lead Acetate**

Tannins are precipitated with lead acetate solution.

* **With Ferric Chloride**

Generally phenols were precipitated with 5% w/v solution of ferric chloride in 90% alcohol and thus phenols are detected.

* **With Gelatin Solution**

To a solution of tannins (0.5-1%) aqueous solution of gelatins (1%) and sodium chloride (10%) were added. A white buff precipitate conform the compounds.

TEST FOR STEROIDS AND STEROLS

* **Salkowski's Test**

In this test to 5 ml of the solution of extract in chloroform in a dry test tube, equal volume of conc. H_2SO_4 was added along the side of the test tube and the upper chloroform layer and lower acid layer were observed. The presence of steroids or sterols are confirmed by the upper layer showing a play of colours first from bluish red to gradually violet and lower acid layer showing yellow colour with green fluorescence.

* **Libermann-Burchard Reagent**

In this method of detection, about 2ml of the solution extract in chloroform was placed in a dry test tube. Then 2ml of acetic anhydride and 2-3 drops of conc. Sulphuric acid was added to it and allowed to stand for few minutes. An emerald green colour develops if steroids or sterols are present.

TEST FOR TRITERPENOIDS

* **Tin and Thionyl Chloride**

For detection of triterpenoids the extract was dissolved in chloroform. A piece of metallic tin and 1 drop of thionyl chloride was added to it. Pink colourconforms the result.

TEST FOR SAPONINS

* **Foam Test**

About 1 ml of alcoholic and aqueous extract was diluted separately with distilled water to make the volume up to 10 ml. And shaken in a graduated cylinder for 15 min. and kept aside. 1cm layer of foam after standing for 30 min. indicates the presence of saponins.

TEST FOR FLAVONOIDS

* **With NaOH**

For the detection of flavonoids, the extract was first dissolved with water. It was filtered and the filtrate was treated with sodium hydroxide. A yellow colourconforms the presence of flavonoids.

* **With Sulphuric Acid**

A drop of sulphuric acid when added to the above, the yellow colour disappears.

* **With Mg & HCL**

In this method of detection, the extract to be tested was dissolved in water. It was then filtered and the filtrate with magnesium. After that, a few drops of conc. HCL was added to it. A pink colour confirmed the presence of flavonoids.

The evaluation of Preliminary phytochemical tests for presence of secondary phytoconstituents is tabulated in the table.

C: PHARMACOLOGICAL STUDY

Evaluation of anthelmentic activity of ethanolic extract of *(Indian Kamala)* experimental animals

Objective: The aim of the present study was to evaluate the anthelmentic property of ethanolic extract of

(*Indian Kamala)* in and MES convulsions in experimental models

Assessment of the Larvicidal activity

Evaluation of *invitro* anthelmintic activity

Experimental procedure

Experimental worms

All the experiments were carried out in Indian adult earthworms (*Indian Kamala)* due to its anatomical resemblance with the intestinal roundworm parasites of human beings. They were collected from moist soil and washed with water to remove all fecal matters. [38]

Preparation of extracts

The plant leaf powder was subjected to hot water maceration to obtain aqueous extracts. The dry powder is extracted with benzene and methanol using maceration process for 48 hours. The powdered (*Indian Kamala)* was extracted exhaustively with increasing polarity solvents (methanol, benzene and water) for 72 hours followed 48 hours and 24 hours. The solvents were pooled, distilled under vaccum and dried under vaccum dessicator. Different concentrations (25, 50, 100 and 200mg) of each extract solution were prepared by diluting the stock solution, in propylene glycol, using normal saline.

5. RESULTS & DISCUSSION

PHARMACOGNOSTICAL INVESTIGATION

Organoleptic Evaluation

Table 2: Organoleptic evaluation of the plant (Indian Kamala)

Aerial part	
Characters	**Observation**
Colour	Reddish brown
Texture	Coarse
Taste	Bitter
Odour	Odourless

Powder microscopy

Table 3: Powder microscopy of the aerial part of the plant. (*Indian Kamala)*

SI.NO.	Part of the plant	Characters
1.	Dry powder of the aerial part of (*Indian Kamala)*	Uniseriate multicellular trichomes Anisocytic stomata cruciferous Sclerenchymatus fibers Starch grains Bundle of acicular Cluster crystals

Stomatal number

Table 4: Stomatal number of the plant (*Indian Kamala)*

SI NO.	Parameters	Value (1mm^2)
1	Vein islet number (1 mm2 leaf surface)	22
2	Vein termination number	15
3	Stomatal index (per sq.mm)	Upper surface-0.22 Lower surface- 035
4	Stomatal number (per sq.mm)	Upper surface-15.94 Lower surface-27.09

PHYSICO-CHEMICAL INVESTIGATION

Foreign organic matter

Table 5: foreign organic matter of the plant (*Indian Kamala)*

SI. NO.	Parameter	% yield (w/w)
1	Foreign Organic matter	0.18

Ash value

Table 6: Ash value of the plant (*Indian Kamala)*

SI.NO.	Parameters	% Values (w/w)
1	Total ash	27-37%
2	Acid insoluble ash	37%
3	Water soluble ash	11-16%

Moisture content (Loss on drying)

Table 7: Moisture content of the plant (*Indian Kamala)*

SI.NO.	Parameter	%Value (w/w)
1	Moisture content	1.74

Swelling index

Table 8: swelling index of the plant (*Indian Kamala)*

SI.NO.	Parameter	Value (cm.)
1	Swelling index	0.8

Foaming index

Table 9: foaming index of the plant (*Indian Kamala)*

SI.NO.	Parameter	Value
1	Foaming index	Less than 100

PHYTOCHEMICAL INVESTIGATIONS

Extractive Yield of Different Extracts

Table 10: Extractive values of the (*Indian Kamala)*

Extracts	**% Yield**	**Color**
Petroleum Ether	4.9%	Reddish brown
Chloroform	5.45%	Reddish brown
Ethyl acetate	9.77%	Reddish brown
Ethanol Extract	8.75%	Reddish brown
distilled water	4.4%	Reddish brown

Table 11: The phytoconstitutent of the plant (*Indian Kamala)*

Sl. No.	Test/ reagent used	Extracts		
		Pet. ether extract	Chloroform extract	Ethanol Extract
1	**Alkaloids**			+
	Mayer's Reagent	-	-	+
	Dragendroff's Reagent	-	-	+
	Wagner's Reagent	-	-	+
	Hager's Reagent	-	-	+
2	**Carbohydrates:-**			
	Molisch's Test	-	-	+
	Fehling's Test	-	-	+
	Benedict's Reagent	-	-	+
	Barfoid's Test	-	-	+
	Iodine Test	-	-	+
3	**Glycosides:-**			
	Keller-Killiani Test	+	_	_
	Legal Test	+	_	_
	Modified Borntrager's Test	+	_	_
	Borntrager's Test	+	_	_
4	**Proteins and Amino acids:-**			
	Ninhydrine Test	_	_	+
	Biuret Test	_	_	+
	Millon's Test	_	_	+
	Xanthoproteic Test	_	_	+
5	**Tannin:-**			
	Ferric chloride solution	_	+	+
	Gelatin solution	_	+	+
	Lead acetate solution	_	+	+
6	**Terpenoids**	+	+	-
7	**Saponin**	+	_	_
	Foam Test	+	_	_
	With $NaHCO_3$	+	_	_
8	**Flavonoids**			
	With NaOH	_	_	+
	With H_2SO_4	_	_	+
	With Mg/HCl	_	_	+
9	**Steroids:-**			
	Liebermann's Test	_	+	+
	Salkowski test	_	+	+

(+) Sign for present and (-) Sign for absent.

PHARMACOLOGICAL STUDY

Evaluation of in vitro anthelmintic activity

Experimental procedure

Experimental worms

All the experiments were carried out in Indian adult earthworms (Indian Kamala) due to its anatomical resemblance with the intestinal roundworm parasites of human beings. They were collected from moist soil and washed with water to remove all fecal matters.

Preparation of extracts

The plant leaf powder was subjected to hot water maceration to obtain aqueous extracts. The dry powder is extracted with benzene and methanol using maceration process for 48 hours. The powdered (Indian Kamala) was extracted exhaustively with increasing polarity solvents (methanol, benzene and water) for 72 hours followed 48 hours and 24 hours. The solvents were pooled, distilled under vaccum and dried under vaccum dessicator. Different concentrations (25, 50, 100 and 200mg) of each

extract solution were prepared by diluting the stock solution, in propylene glycol, using normal saline.

Standard drug - albendazole

Albendazole was prepared by dissolving them in normal saline at a concentration of 15mg/ml.

Experimental control treatment

A 10% propylene glycol in normal saline was used as experimental control treatment.

Normal control

Saline was prepared and used to treat the normal control group.

Administration of extract

Different concentrations (25, 50, 100 and 200mg) of each extract solution were prepared by diluting the stock solution, in propylene glycol, using normal saline and final volume was made up to 10 ml for respective concentration. Albendazole was used as standard. Groups of approximately equal size worms consisting of four earthworms individually in each group were released into in each 10 ml of desired concentration of drug and extracts in the petridish.

Experimental design

The anthelmintic activity was performed according to the Ghosh et al., method.[25] On adult Indian earth worm (Indian Kamala) as it has anatomical and physiological resemblance with the intestinal round worm parasites of human beings. (Indian Kamala) was placed in petridish containing four different concentrations (25, 50, 100 and 200mg) of methanolic & aqueous extract of (Indian Kamala). Each petridish was placed with 4 worms and observed for paralysis or death. Mean time for paralysis was noted when no movement of any sort could be observed, except when the worm was shaken vigorously; the time death of worm (min) was recorded after ascertaining that worms neither moved when shaken nor when given external stimuli. The test results were compared with reference compound albendazole (15 mg/ml) treated samples.

6. RESULTS

Table 12- indicates the phytochemical constituents of methanolic, benzene and aqueous extract of the (Indian Kamala) when subjected to qualitative analysis for carbohydrates, protein, alkaloids, flavonoids, steroids, saponin, glycosides, terpen oids, tannins and phenols. By preliminary phytochemical screening it was found that all the three extract of plant contain carbohydrates, protein, alkaloids, flavonoids, steroids, saponin, glycosides, terpenoids, phlobatannins, tannins and phenols.

Table 13**s**hows higher concentration of extract produced paralytic effect much earlier and time taken for death was shorter for worms. Aqueous and methanol extract of (*Indian Kamala)* exhibited anthelmintic activity in dose – dependent manner showing maximum efficacy at 25, 50, 100 and 200, mg/ml concentration for worms than benzene extract of (*Indian Kamala)*

Figure 13- shows higher concentration of extract produced paralytic effect much earlier for worms. Aqueous and methanol extract of (*Indian Kamala)* exhibited anthelmintic activity in dose- dependent manner showing maximum efficacy at 25, 50, 100 and 200 mg/ml concentration for worms than benzene extract of(*Indian Kamala).*

Figure 13- shows higher concentration of extract time taken for death was shorter for worms. Aqueous and methanol extract of (*Indian Kamala)* exhibited anthelmintic activity in dose- dependent manner showing maximum efficacy at 25, 50, 100 and 200 mg/ml concentration for worms than benzene extract of (*Indian Kamala)*

Table 12: Phytochemical alalysis of different solvent extracts of *(Indian Kamala)*

S.No	Phytochemical constituents	Methanolic extract	Benzene extract	Aqueous Extract
1.	Carbohydrate	+	+	+
2.	Protein and amino acids	+	+	+
3.	Alkaloids	+	+	+
4.	Flavonoids	+	+	+
5.	Steroids	+	+	+
6.	Saponin	+	+	+
7.	Tannins	+	+	+
8.	Phenols	+	+	+
9.	Glycosides	-	+	+

(+) Present, (-) Absent

Table 13: *In vitro* anthelmintic activity of various extracts *(Indian Kamala)*

Group	Treatment of extracts	Concentration (mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
1.	Normal control	-	-	-
2.	Experimental control	-	-	-
3.	Albendazole	15	1.60±43	1.60±55
4.	Methanol	25	4.16±58	6.35±74
		50	4.54±52	2.94±63
		100	2.38±33	2.64±42
		200	1.63±27	2.00±35
	Hydroalcololic	25	08.9±77	08.9±97
		50	47.3±64	47.3±81
		100	36.1±53	36.1±69
		200	28.1±38	28.1±55
6.	Aqueous	25	9.91±56	9.91±69
		50	9.72±50	9.72±60
		100	1.29±42	1.41±48
		200	1.29±27	1.41±32

SD; n=4 in each group. Comparisons made between standard±All values represents mean versus treated groups.

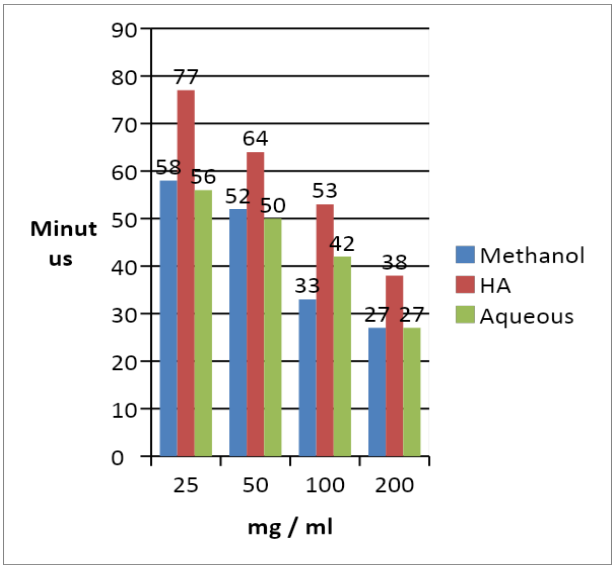

Figure 18: Time taken for paralysis of Pheretima posthuma by various solvent extracts of *(Indian Kamala)*

7. DISCUSSION

Helminth infections are among the most widespread infections in humans, distressing a huge population of the world. Although the majority of infections due to helminths are generally restricted to tropical regions and cause enormous hazard to health. To evaluate compounds with anthelmintic activity, a number of substances were analyzed using different species of worms, for example, earthworms, Ascaris, Nippostrongylus and Heterakis. From all these species, earthworms have been used extensively for the preliminary evaluation of anthelmintic compounds invitro because they are similar to intestinal "worms" in their reaction to anthelmintics and are easily accessible. It has been verified that all anthelmintics

Which are toxic to earthworms are creditable to study as an anthelmintic.[26] Earthworms have the ability to move by ciliary movement. The outer layer of the earthworm is a mucilaginous layer and composed of complex polysaccharides. This layer being slimy enables the earthworm to move freely. Any damage to the mucopolysaccharide membrane will expose the outer layer and this restricts its movement and can cause paralysis. This action may lead to the death of the worm by causing damage to the mucopolysaccharide layer. This causes irritation leading to paralysis. Commonly used anthelmintic drugs like piperazine citrate and albendazole by increasing chloride

ion conductance of worm muscle membrane produces hyper polarization and reduced excitability that leads to muscle relaxation and flaccid paralysis.

8. SUMMARY

The major challenge in natural product drug discovery is to determine the number of active samples pursue for further studies, usually for isolation and structure elucidation of the active constituents since isolation and structure elucidation may be the most laborious, time consuming & expensive in natural product drug discovery. So much attention has to be given to develop reliable method. Now compound that show promising therapeutic potential on biological activity are subject to further study aimed at developing a promising pharmaceutical candidate for preclinical & clinical trials. Once a drug candidate has been selected for development, these are no different between a natural products derived new drug candidate & synthetically derived/ designed drug candidate in terms of the process for further development. But sufficient amount required for clinical studies are only fulfilled through semi synthesis, re isolation of wild or cultivated plants or by tissue culture scale up. Although the use of natural products to treat or control disease is well documented by biochemical basis of observed efficacies was established when a number of important pharmaceutically active natural products were discovered. Many of these biological active natural products also help as a biochemical probes to unravel the principles of human pharmacology.

From the performance I had evaluated many physic-chemical parameters and summarized the value. The organoleptic character reported that the plant was green in colour, coarse in texture taste was characteristic and has a pleasant odour. In powder microscopy it was found that uniseriate multicellular trichomes, anisocytic stomata, cruciferous, Sclerenchymatus fibers, starch grains, bundle of acicular and cluster crystals. The stomatal number, stomatal index, vein isolate number and vein termination number evaluated. Finally the foreign organic matter, ash value, moisture content, swelling index, foaming index was determined and the value was 0.18%w/w,27-37%w/w,1.74%w/w,0.8cm, less than 100 respectively. The phytochemical determination was reported as petroleum ether 4.9%, chloroform 5.45%, ethyl acetat 9.77% and ethanol 8.75%. The primary & secondary metabolites were terpenoids was present in pet. Ether extract, in chloroform extracts terpenoids and steroids were present, whether carbohydrate, glycoside, tannin, flavonoids and steroids were present in ethanolic extracts. The therapeutic value reported that it has an anthelmentic effect in ethanolic extract and significant at a high dose of 120mg/kg by is show the good pharmacological activity.

9. CONCLUSION

The process of new drugs development is enforced by the success of herbal drugs in pharmaceutical market. Another approach to natural product drug discovery is to utilize the information derived from traditional system that have used plant product to control disease and injury. From industrial view point regarding sufficient supply of natural product derived active constituents. As the amount of secondary metabolites are less so the yield are less. One approach that has been successful in development of simpler semi-synthetic or synthetic analogs is designed which also improves its pharmaceutical properties. A great approach to meet the demand of secondary metabolites is the use of tissue culture techniques.

From the experimental work it was clear that the Aqueous shows positive test for terpenoids , Methanolic shows to contain terpenoids and steroids, chloroform extract shows positive test for carbohydrate, glycosides and steroids where as ethanol extract found to contain carbohydrate, cardiac glycosides, tannins, flavonoids and saponins. The therapeutic value reported that it has an anthelmentic effect in ethanolic extract and significant at a high dose of 200mg/ml by is show the good pharmacological activity.

10. ACKNOWLEDGEMENT

My heartfelt thanks to Ms. Shipra, Dr.R.K Singhal Mr. Ajay kumar and Mr. Vidhan Singhal for funding myresearch project and utilization for different instrumentsand equipment in Department of Pharmacognosy andDepartment of Pharmacology, college of Pharmacy, Keshav Pharmacy College Muzaffarnagar U.P..

REFERENCES

[1] Kritikar KR, Basu BD.Indian Medicinal Plants.International Book Distributers Dehradun India.YNM; 3:2266-2270.
[2] Sharma J, Varma R. A Review on Endangered plant of (Indian Kamala) Mallotus philippensis (Lam.) Pharmacologyonline. 2011; 3: 1256-1265.
[3] Said H. Hamdard Pharmacopoeia of Eastern Medicine. Times Press, Pakistan.1970; 400.
[4] https://en.wikipedia.org/wiki/Mallotus_philippensis. Cited on 14-07-2015.
[5] https://www.google.co.in/search?q=taxonomy+of+Mallotu.
[6] Gupta SC, Gupta SS, Agarwal JS. The component acids of kamala oil(Indian Kamala) (Mallotus Philippinensis, Muell. Arg.). Journal of the American Oil Chemists Society.1954; 31(7); 287-289.

[7] Nadkarni AK.Indian Materia Medica. Popular Parkashan. YNM; 1:760-763.
[8] GangawarM,GoelRR,NathG(Indian Kamala).Mallotus philippinensis Muell. Arg (Euphorbiaceae): Ethnopharmacology and Phytochemistry Review. BioMed Research International.2014.
[9] Adhav M.Phytochemical screening and antimicrobial activity of(Indian Kamala) Mallotus philippensis Muell. Arg. Journal of Pharmacognosy and Phytochemistry. 2015; 3(5): 188-191.
[10] Khare CP. Indian Medicinal Plants. An Illustrated Dictionary. Published by Springer. 2007; 393-394.
[11] Khan M, Qureshi RA, Hussain M**,** Mehmood K, Khan R**.** Hexane soluble extract of (Indian Kamala) Mallotus philippensis (Lam.) Muell. Arg. root possesses anti-leukaemic activity Chemistry Central Journal. 2013, **7**:157.
[12] Anonymous. The Wealth of India. CSIR. New Delhi.2009; 6(L-M):229-232.
[13] Panthi Mp, Singh Ag. Ethnobotany of Arghakhanchi District, Nepal: Plants Used In Dermatological and Cosmetic Disorders. Int J Appl Sci Biotechnol.2013; 1(2): 27-32.
[14] Hakim MAH. Bustanul Mufradat Jadeed. Idara Kitab Ul Shifa New Delhi. 2002:453-454.
[15] Ghani N. Khazainul Advia. Idara Kitab Ul Shifa. New Delhi. 2010:1067-1068.
[16] Anonymous. Qarabadine Majeedi. Hamdard Wakf Lab. 1986:231.
[17] Anonymous. Qarabadine Azam. Aejaz Publishing House New Delhi. 1996:453.
[18] Anonymous. Qarabadine Jadeed.Central Council for Research in Unani Medicine. New Delhi. YNM: 137-138.
[19] Kabeeruddin HM. Biyaze Kabir. Hikmat Book Depo Hyderabad India. YNM: 1:90.
[20] Satyavati G. V, Gupta K. A and Tandon N .1987. Medicinal Plants of India Volume 2. New Delhi: Indian Council of Medical Research: 201-206.
[21] Gupta A K, Chauhan J. S. 1984. Constituents from the stem of Bauhinia variegate. Nat Acad Sci Lett .**,7**:15-16.
[22] Lounasmaa M, Widen C. J, Tuuf C. M and Huhtikangas A. 1975. On the phloroglucinol derivatives of Mallotus philippinensis. (Indian Kamala) Planta Med., **28:**16-31.
[23] Usmanghani K, Saeed A and Alam M . T. 1997. Indusynic Medicine., Karachi: Research Institute of Indusyunic Medicine**:** 285-287.
[24] Zabihullah O, Rasheed A and Akhter N. 2006. Ethnobotanical survey in Kot Manzaray Baba valley Pakistan**.** J Plant Sci., **12**: 115-121.
[25] Iqbal Z, Lateef M, Ashraf M and Jabbar, A. 2004. Anthelmintic activity of Artemisia brevifoliain sheep. J. Ethnopharmacol., **93:** 265–268.
[26] Coles, G. .C.; Jackson, F.; Pomroy, W.E.; Prichard, R.K.; von Samson-Himmelstjerna, G.; Silvestre, A.; Taylor, M.A.; and Vercruysse, J. 2006. The detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol. **136** : 167–185.
[27] Rahman, W.A., Lee, R. and Sulaiman, S.F. 2011. Invitro Anthelmintic activity of Neem Plant (Azadirachta indica) extracts against third stage Haemonchus contortus larvae from goats. Global veterinaria **7(1):**22-26.
[28] Githiori J.B Athanasiadou, S and Thamsborg, S.M 2006. Use of plants in novel approaches for control of gastrointestinal helminths in livestock with emphasis on small ruminants. Vet. Parasitol.,**139:** 308–320.
[29] Priya, M.N.; Sreeshitha, S.G.; Sreedevi, R.; Sujith, S.; Deepa, C.K.; Suja R.S. and Juliet, S. 2014. Anthelmintic activity of different extracts of (Indian Kamala) Mallotus phillipensis invitro. Life Sci. Int. Res J. **1:** 152-155.
[30] Athanasiadou S, I.; Kyriazakis, F.; Jackson and Coop, R.L. 2001. Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: in vitro and in vivo studies. Vet Parasitol. **99:** 205-219.
[31] Hrčkova, G and Velebný, S. 2010. Flavonoid silymarin potentiates antihelmintic effect of praziquantel via down-regulation of oxidative stress and fibrogenesis in the liver. Proceedings of the World Medical Conference 250-257.
[32] Azando, E.V.B; Hounzangbe-Adote, M.S.; Olounlade, P.A.; Brunet, S and fabre, N. 2011. Involvement of tannins and flavonoids in the invitro effects of Newbouldia laevis and zanthoxylum zanthoxyloides extracs on the exsheathment of third stage infective larvae og gastrointestinal nematodes. Vet. Parasitol.**180**: 292-297.
[33] Williams, A.R., Ropiak, H.M., Fryganas, C., Desrues, O., Muller-Harvey, I and Thamsborg, S.M. 2014. Assessment of the anthelmintic activity of medicinal plant extracts and purified condensed tannins against free-living and parasitic stages of Oesophagostomum dentatum. Parasites vectors **7**: 518-530.
[34] Hernandez-Villegas, M.M., Borges-Argaez, P., Rodriguez-Vivas, R.I., Torres-Acosta, J.F.J., Merndez-Gonzalez, M. and Cacers-Farfan, M. 2011. Ovicidal and larvicidal activity of the crude extracts from Phytolacca icosandra against Haemonchus contortus. Vet. Parasitol. 179: 100-106.
[35] Kalia AN, Textbook of Industrial Pharmacognosy, 1st Reprint Edn, CBS Publishers & Distributors: 36 – 37, (2009).
[36] Guidelines for the regulation of Herbal medicines in South east Asia Region, World Health Organisation, Regional office, New delhi, 1st Edn: 1, (2004).
[37] Rasheeduz Zafar, Medicinal Plants of India, 6st Reprint Edn, CBS Publishers & Distributors: 89 – 91, (2006).
[38] R.N.Chopra, I.C.Chopra and B.S.Varma, Supplement to Glossary of Indian Medicinal Plants, Reprint Edn, National Institute of Science Communication, 63, (1998).
[39] Kirtikar KR, Basu BD, Indian Medicinal Plants, 2nd Edn, Vol 3, International Book Distributors: 2268, (1984).
[40] Quality Control Methods for Medicinal Plant Material. World Health Organisation: Geneva, 1st Edn: 4–73, (1996).
[41] Pulok Mukherjee K, Quality Control of Herbal Drugs, 1st Reprint Edn, Business Horizons Pharmaceutical Publishers: 132 – 178, 426 – 458, (2005).
[42] Indian Pharmacopoeia, 3rd Edn: Vol 2, The Controller of Publications: New Delhi, A-74, A-86, (1996).
[43] The Ayurvedic Pharmacopoeia of India, 1st Edn, Vol 2, The Controller of Publications: New delhi, 187 – 196, (1999).
[44] Padmavathi D, Susheela L and Vijaya Bharathi K, Pharmacognostical evaluation of Barringtonia acutangula leaf: Int J Ayurveda Res, 2 (1): 37 - 41, (2011).
[45] Patil AG, Koli SP, Patil DA and Naresh Chandra, Pharmacognostical standardization and HPTLC fingerprint of Crataeva tapia Linn. ssp. odora (Jacob.) Almeida leaves: Int J Pharm Bio Sci, V1 (2): 1 – 14, (2010).
[46] Brain KR, Turner TD, The Practical Evaluation of Phytopharmaceuticals, Wright-Scientechnica: 4–9, (1975).
[47] Sass JE, Elements of Botanical Microtechnique, McGraw Hill Book Co: 222, (1940).
[48] Johanson DA, Plant Microtechnique, McGraw Hill Book Co: 182-185,523, (1940).

[49] O'Brien TP, Feder N, Mc Cull ME, Polychromatic staining of plant cell walls by toluidine blue-O, Protoplasma, 59: 368 – 373, (1964).
[50] Trease and Evans WC, Pharmacognosy, 15th Edn, Saunders: 545–547, (2003).
[51] Khandelwal KR, Practical Pharmacognosy, 18th Edn, Nirali Prakashan: 146 – 161, (2007).
[52] Kokate CK, Practical Pharmacognosy, 4th Reprint Edn, Vallabh Prakashan: 107-111, (2005).
[53] Sadasivam S and Manickam A, Biochemical Methods, 2nd Edn, New Age International Publishers: 1 – 200, (1996).
[54] Edeoga HO, Okwu DE and Mbaebie BO, Phytochemical constituents of some Nigerian medicinal plants: Afr J Biotechnol, 4 (7): 685-688, (2005).
[55] Harbone JB, Phytochemical methods, 3rd Edn, Chapman and Hall: 49-244, (1998).
[56] Madhavan V, Chandra Nagar J, Mythreyi R, Gurudeva MR and Yoganarasimhan SN, Pharmacognostical studies on the adventitious roots of Pandanus fascicularis Lam. (Pandanaceae): Asian journal of traditional medicines, 4 (2): 58 – 65, (2009).
[57] Nazneen Bobby MD, Wesely EG and Johnson M, High performance thin layer chromatography profile studies on the alkaloids of Albizia lebbeck: Asian Pac J Trop Biomed, S1 – S6, (2012).
[58] Kataria S, Kamalaksha Rao S, Anil Bhandari and Dilsher Kaur, Pharmacognostical standardization of Corchorus depressus (L.) Stocks (Tiliaceae) – A promising ethnomedicinal plant: Indian Journal of Traditional Knowledge, 12 (3): 489 – 497, (2013).
[59] Anastassiades M, Lehotay SJ, Stajnbaher D and Schenck FJ, Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and "Dispersive Solid-Phase Extraction" for the Determination of Pesticide Residues in Produce: J. AOAC Int, 86(2): 412 – 431, (2003).
[60] "QuEChERS Methodology: AOAC Method". Accessed on "20 January 2012" . http: //www.restek.com / pdfs / 805-01-002. pdf.
[61] Lehotay SJ, Mastovska K, Lightfield AR, Use of Buffering and Other Means to Improve Results of Problematic Pesticides in a Fast and Easy Method for Residue Analysis of Fruits and Vegetables: J. AOAC Int, 88(2): 615 – 629, (2005).
[62] Official Methods of Analysis, 18th Edn. AOAC Internat C.-J. Widen´ and H. S. Puri, "Natural occurrence and chemical variability of phloroglucinols in Kamala," Planta Medica, vol. 40, no. 3, pp. 284–287, 1980.
[63] R. Singh, K. C. Singhal, and N. U. Khan, "Antif ilarial activity of (Indian Kamala) Mallotus philippinensis Lam. on Setaria cervie (Nematoda: Filar-ioidea) in-vitro," Indian Journal of Physiology and Pharmacology, vol. 41, no. 4, pp. 397–403, 1997.
[64] V. P. Kumar, N. S. Chauhan, H. Padh, and M. Rajani, "Search for antibacterial and antifungal agents from selected Indian medicinal plants," Journal of Ethnopharmacology, vol. 107, no. 2, pp. 182–188, 2006.
[65] S. Shrikumar and T. K. Ravi, "Approaches towards development and promotion of herbal drugs," Pharmaceutical Reviews, vol. 1, no. 1, pp. 180–183, 2007.
[66] C. Orwa, A. Mutua, and R. Kindt, "Agroforestree Database: a tree reference and selection guide," Version 4. 0, 2009.
[67] K. Usmanghani, A. Saeed, and M. T. Alam, "Indusynic Medicine. Karachi," Research Institute of Indusyunic Medicine, vol. 1997, pp. 285–287, 1997.
[68] F. Ahmad and S. Hashmi, "Adulteration in commercial Kamila(Indian Kamala) (Mallotus philippinensis Muell.) an anthelmintic drug of repute," Hamdard Medicus, vol. 38, pp. 62–67, 1995.
[69] K. D. Roberts, E. Weiss, and T. Reichstein, "Glycosides and aglycons. CCLII. Cardenolides of the seed of (Indian Kamala) Mallotus philippinensis," Helvetica Chimica Acta, vol. 46, pp. 2886–2893, 1963.
[70] M. Bandopadhyay, V. K. Dhingra, and S. K. Mukerjee, "Triter-penoid and other components of(Indian Kamala) Mallotus philippinensis," Phy-tochemistry, vol. 11, no. 4, p. 1511, 1972.
[71] S. P. Nair and J. Madhusudana Rao, "Kamaladiol-3-acetate from the stem bark of (Indian Kamala) Mallotus philippinensis," Phytochemistry, vol. 32, pp. 407–409, 1993.
[72] J. D. Wansi, J. Wandji, W. A. Kamdem et al., "Triterpenoids from Drypetes chevalieri Beille (Euphorbiaceae)," Natural Product Research, vol. 20, no. 6, pp. 586–592, 2006.
[73] P. Castenada, A. Bahena, and E. Garcia, "Chemical studies on Mexican plants used in traditional medicine, XXIX. Sec-ondary metabolites from the stem bark of Celaeno-dendron mexicanum," Journal of Natural Products, vol. 56, pp. 1575–1579, 1993.
[74] T. Tanaka, T. Ito, M. Iinuma, Y. Takahashi, and H. Naganawa, "Dimeric chalcone derivatives from (Indian Kamala) Mallotus philippinensis," Phytochemistry, vol. 48, no. 8, pp. 1423–1427, 1998.
[75] T. M. Nguyen, V. M. Chau, V. K. Phan et al., "Study on chemical constituents of the leaves of(Indian Kamala) Mallotus philippinensis," Tap Chi Hoa Hoc, vol. 48, pp. 352–357, 2010.
[76] M. Furusawa, Y. Ido, T. Tanaka et al., "Novel, complex flavonoids from(Indian Kamala) Mallotus philippinensis (Kamala tree)," Helvetica Chimica Acta, vol. 88, no. 5, pp. 1048–1058, 2005.
[77] M. Lounasmaa, C. J. Widen, C. M. Tuuf, and A. Huhtikangas, "On the phloroglucinol derivatives of (Indian Kamala) Mallotus philippinensis," Planta Medica, vol. 28, no. 1, pp. 16–31, 1975.
[78] S. F. H. Zaidi, I. Yoshida, F. Butt et al., "Potent bactericidal con-stituents from(Indian Kamala) Mallotus philippinensis against clarithromycin and metronidazole resistant strains of Japanese and Pakistani helicobacter pylori," Biological and Pharmaceutical Bulletin, vol. 32, no. 4, pp. 631–636, 2009.
[79] M. Arfan, H. Amin, M. Karamac et al., "Antioxidant activity of extracts of (Indian Kamala) Mallotus philippinensis fruit and bark," Journal of Food Lipids, vol. 14, no. 3, pp. 280–297, 2007.
[80] M. Arfan, K. Hazrat, and K. Magdalena, "Antioxidant activity of phenolic fractions of (Indian Kamala) Mallotus philippinensis bark extract," Journal of Food Science, vol. 27, no. 2, pp. 109–117, 2009.
[81] R. Saijo, G. Nonaka, and I. Nishioka, "Tannins and related com-pounds. LXXXVIII. Isolation and characterization of hydrolyz-able tannins from (Indian Kamala) Mallotus japonicus (Thunb.) Mueller-Arg. And M. philippinensis (Indian Kamala) (Lam.) Mueller-Arg," Chemical & Pharmaceu-tical Bulletin, vol. 37, no. 11, pp. 2940–2947, 1989.
[82] S. C. Gupta, S. S. Gupta, and J. S. Aggarwal, "Chemical exami-nation of the seeds of (Indian Kamala) Mallotus philippinensis. III. Constitution of kamlolenic acid isolated from the oil," Journal of Scientific & Industrial Research, vol. 12, pp. 240–242, 1953.
[83] S. C. Thakur, S. S. Thakur, S. K. Chaube, and S. P. Singh, "An etheral extract of Kamala (Indian Kamala) (Mallotus philippinensis (Moll. Arg) Lam.) seed induce adverse effects on reproductive parameters of (Indian Kamala) female rats," Reproductive Toxicology, vol. 20, no. 1, pp. 149– 156, 2005.
[84] M. L. Gujral, D. R. Varma, K. N. Sareen, and A. K. Roy, "Oral contraceptives. II. Antifertility effect of(Indian Kamala) Mallotus philippinensis," Indian Journal of Medical Research, vol. 48, pp. 52–58, 1960.
[85] K. Moorthy, K. Srinivasan, C. Subramanian, C. Mohanasundari, and M. Palaniswamy, "Phyto-chemical screening and antibac-terial evaluation of stem bark of (Indian Kamala) Mallotus philippinensis var.

Tomentosus," African Journal of Biotechnology, vol. 6, no. 13, pp. 1521–1523, 2007.

[86] M. Gangwar, D. Kumar, R. Tilak et al., "Qualitative phy-tochemical characterization and antibacterial evaluation of glandular hairs of (Indian Kamala) Mallotus philippinensis fruit extract," Journal of Pharmacy Research, vol. 4, no. 11, pp. 4214–4216, 2011.

[87] A. Daikonya, S. Katsuki, and S. Kitanaka, "Antiallergic agents from naturalsources 9. Inhibition of nitric oxide production by novel chalcone derivatives from (Indian Kamala) Mallotus philippinensis

[88] (Euphorbiaceae)," Chemical & Pharmaceutical Bulletin, vol. 52, no. 11, pp. 1326–1329, 2004.

[89] M. Gschwendt, H. J. Muller, K. Kielbassa et al., "Rottlerin, a novel protein kinase inhibitor," Biochemical and Biophysical Research Communications, vol. 199, no. 1, pp. 93–98, 1994.

[90] Y. F. Liao, Y. C. Hung, W. H. Chang et al., "T he PKC delta inhibitor, rottlerin, induces apoptosis of haematopoietic cell lines through mitochondrial membrane depolarization and caspases'cascade," Life Sciences, vol. 77, no. 6, pp. 707–719, 2005.

[91] H. S. Kang, E. K. Park, K. H. Kim et al., "Receptor activator of nuclear factor-kappa B is induced by a rottlerin-sensitive and p38 MAP kinase-dependent pathway during monocyte differentiation," Moleculess and Cells, vol. 17, no. 3, pp. 438–445, 2.