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Review Article

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Butea monosperma Lamkuntz. and *Indigofera tinctoria* Linn. - Standardization with Phytopharmacological Review

Manoj Tripathi[1] and Raghvendra Soni[2]*

[1]Senior Research Officer and [2]Young Scientist, Deendyal Research Institute (Chitrakoot) M.P., India

*Corresponding Author E-mail: sonir0872@gmail.com

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ABSTRACT

Ayurveda research gives lot of experience to determine imbalances of three chemical substances in our body (vatta, pitta, kapha) these three chemicals imbalance is known as tridosha , and various types of diseases are occur due to create imbalance tridosha in our body,herbal medicine research in ayurveda can create motive and The materia medica of the Ayurveda, composed of the five basic elements, has been categorised according to the derivatives of these elements. They include; taste (rasa), potency (virya), taste of the digestion product (vipaka), properties (guna) specific properties (prabhava) and action (Karman). Finally the action (karman) of a drug on the body is expressed in terms of the three doshas. A drug can increase or decrease the vata dosha, the pitta dosha and the kapha dosha.as useful for determine to cure these tridosha, research chalanges to ensure quality, safety of drug according to tridosha in our different organs of body, to discover new herbal medicine(eg. Butea monosperma and Indigofera tinctoria) is opportunities to deal with imbalance of tridosha and these imbalance is control as per drug decoction quantity,this shows efficacy of drug,which is determine by cureness activity of tridosha imbalance in our body.present research work shows standardization aspects of these two herbel medicine these are- Physicochemical tests, Phytochemical tests,Microscopical visualisation of cells/ tissues HPTLC,Microbial culture tests.

Key words: *Butea monosperma, Indigoferatinctoria, Materiamedica, HPTLC*

INTRODUCTION

"Standardization expression is used to describe all measures, which are taken during the manufacturing process and quality control leading to a reproducible quality, It also encompasses the entire field of study from birth of a plant to its clinical application and it is the process of developing and implementing technical standards." The goals of standardization can be to help with independence of single suppliers (commoditization) compatibility interoperability safety, repeatability, or quality.

"Evaluation" of a drug means confirmation of its identity and determination of its quality and purity and detection of its nature of adulteration. Standardization of herbal drug is not an easy task as numerous factors influence the bioefficacy and reproducible therapeutic effect.

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In order to obtain quality oriented herbal products, care should be taken right from the proper identification of plants, season and areas of collection and their extraction and purification process and rationalizing the combination in case of polyherbal drugs.

CURRENT STATUS

Up to 80% of people in India use either Ayurveda or other traditional medicines. In 1970 the Indian Medical Central Council Act which aims to standardize qualifications for Ayurveda and provide accrediated institutions for its study and research.In India, over 100 colleges offer degrees in traditional Ayurvedic medicine. The Indian government support research and teaching in Ayurveda through many channels at both the national and state levels,and helps institutionalize traditional medicine so that it can studied in major towns and cities.

HOW DO HERBS WORK

In many cases, scientists aren't sure what specific ingredient in a particular herb works to treat a condition or illness. Whole herbs contain many ingredients, and they may work together to produce a beneficial effect. Many factors determine how effective an herb will be. For example the type of environment (climate, insects/worm, soil profile) in which a plant grew will affect it, as well how and when it has harvested and processed.

HOW ARE HERBS USED

The use of herbal supplements has increased dramatically over the past 30 years. Herbal supplements are classified as dietary supplements by the U.S. Dietary Supplement Health and Education Act. (DSHEA) of 1994. That means herbal supplements unlike prescription drugs can be sold without being tested to prove they are safe and effective. However, herbal supplements must be made according to good manufacturing practices.

The most commonly used herbal supplements in the U.S. include Echinacea (Echinacea purpurea and related species). St. John's wort (*Hypericum perforatum*), ginkgo (*Ginkgo biloba*), garlic (*Allium sativum*), saw palmetto (*Serenoa repens*), ginseng (Panax ginseng, or Asian ginseng; and Panax quinquefollus, or American ginseng), goldenseal (*Hydrastis canadensis*), valerian (*Valeriana officinalis*), chamomile (*Matricaria recutita*), feverfew (*Tanacetum purthenium*), ginger (*Zingiber officinale*), evening primrose (Oenothera biennis), and milk thistle (*Silybum mariamum*).

Often, herbs may be used together because the combination is more effective and may have favour side effects. Health care providers must take many factors into account, when recommending herbs, including the species and variety of the plant, the plant's habitat, how it was stored and processed, and whether or not there are contaminants (including heavy metals and pesticides).

HERBAL MEDICINE GOOD FOR DISEASE

Herbal medicine is used to treat many conditions, such as asthma, eczema, premenstrual syndrome, rheumatoid arthritis, migraine, menopausal symptoms, chronic fatigue, irritable bowel syndrome, and cancer, among others. Herbal supplements are best taken under the guidance of a trained health care provider. For example, one study found that 90% of arthritic patients use alternative therapies, such as herbal medicine. Be sure to consult with your doctor or pharmacist before taking any herbs.

PHARMACOLOGY

The materia medica of the Ayurveda, composed of the five basic elements, has been categorised according to the derivatives of these elements. They include; taste (rasa), potency (virya), taste of the digestion product (vipaka), properties (guna) specific properties (prabhava) and action (Karman).

Taste (rasa) is six fold : sweet (madhura), sour (amla), salty (lavana), sharp (tikta), bitter (katu) and astringent (kasaya). Each taste is composed of two of the five elements. The condition of the food substances after digestion is also expressed in terms of taste (vipaka); however it can only be sweet, sour or sharp.

The properties (guna) are grouped in 10 pairs, each on complementary to the other heavy and light, cold and hot, fat and dry, slow and sharp, stable and labile, soft and hard, clear and slimy smooth and raw, fine and massive, viscous and liquid.

The potency (virya) of a drug is defined as its capability to express its property. Sometimes potency is grouped in the same way as the property, but for practical reasons, it is usually expressed in terms of hot (ushna) and cold (shita). The specific property (prabhava)distinguishes two drugs that have the same taste, taste after digestion and potency. This might be due to the composition of the drug or the location in the body where the drug acts.

Finally the action (karman) of a drug on the body is expressed in terms of the three doshas. A drug can increase or decrease the vata dosha, the pitta dosha and the kapha dosha.

The drugs used in Ayurveda are made by several processes from vegetable and mineral raw materials. Mostly plant alkaloids are the active ingredients. Obviously barring some chemical changes it is mostly natural derivates. we hope that you will continue to explore Ayurveda to enhance your health and to gain further insights into this miracle we call life.

MATERIALS AND METHODS

1. SELECTION AND IDENTIFICATION OF MEDICINAL PLANTS:

Name of the plant materials/part selected for study :

1. Butea monosperma Lan. Kuntz {Stem Bark}
2. Indigofera Tinctorious Linn. {Root}

These two medicinal plants with their specific parts were selected for validation with special emphasis on quality control as per ISM. After that, these plants were identified specifically by observation of their external features with the help of farmers/ gardeners/local persons.

2. COLLECTION & AUTHENTICATION OF MEDICINAL PLANTS PARTS:

Once selected medicinal plants were identified then all these two plants (Parts) were authenticated for their originality by Expert Taxonomists of the Research laboratory(self) in Ayurveda Sadan, before their collection. After the authentication of all two medicinal plants & their parts were freshly collected properly from different regions of the Chitrakoot (M.P.) and surroundings of the Arogyadham, Chitrakoot (M.P.)

3. PREPARATION OF DRUG SAMPLE:

After the collection of specified parts of the plants, proper drying in room seem light & then in tray dryer at room temperature was done. When drug was dried properly, powered crude drug sample of the collected parts of these two medicinal plant were prepared by using the apparatus like cutter & mixer grinder etc. and then sieved by using the sieve of different size as; 32, 53, 80 & 100. Prepared powdered drugs(sample) were packed in well closed air tight plastic containers and labeled properly. Prepared drug samples should be totally free from foreign bodies or antigen, net content of the prepared samples were checked.

4. DESCRIPTIVE IDENTIFICATION:

Procedure: Microscopic descriptions of tissues were supplemented with micrographs where necessary. Microscopic slides were made by using the little amount of the powdered sample of medicinal plants named as: Butea Monosperma(stembark) 2.Indigofera Tinctoria (Root), Microscopic slides were also prepared for the study of T.S. of these selected parts (Bark, Root) of these medicinal plants. Then prepared microscopic slides were observed with the help of different microscopes named as: Simple microscope with camera Lucida, compound microscope with camera Lucida & electronic microscope with photographic camera. Observation was done under different magnifications, And Photographs of the powder were taken under different magnifications. Descriptive terms anatomical feature had given in the standard anatomy books, API and other reference books. Various photographs of microscopy of the selected parts of all these medicinal herbs were shown separately in result section.

Instruments/Apparatus used: Microscopes, Brush, Slides, Cover slip, Blade, Tissue paper etc.

Chemicals/Staining reagent used: Drug powder, Fresh plant parts (Bark & Root) Glycerin, Pholoroglucinol, Saffranin, Chloral hydrate solution, Caustic alkali Nitric acid, Conc. HCL, Distill water etc.

5. PHYSICOCHEMICAL ANALYSIS:

DETERMINATION OF TOTAL ASH:

APARATUS REQUIRED: Muffle furnace, Silica crucible with cover, Spatula, Desiccators, Analytical Balance.

PROCEDURE: Incinerate about 2-2 gm. accurately weighed of the ground drug in a silica dish at a temp. Not exceeding 450^{o}C until free from carbon, cool and weigh. If a carbon free ash cannot be obtained in this way, exhaust the charred mass with hot water collect the residue on an ash less filter paper and the filtrate, evaporate to dryness and ignite at a temp. Not exceeding 450^{o}C calculate the percentage of ash with reference to the air.

FORMULA : Average Value X 100/sample

DETERMINATION OF ACID INSOLUBLE ASH:

APPARATUS REQUIRED : Muffle Furnace, Silica crucible with cover, Spatula, Desiccator, analytical Balance.

PROCEDURE:

Boil the ash obtained from total ash value for 5 min. with 25ml of dilute Hcl collect the insoluble matter in a Silica crucible and on an ash less filter paper wash with hot water and ignite to constant weight, calculate the percentage of ash with reference to the air dried drug.

FORMULA : Acid insoluble ash = (m_2-m_1) x $100/m_1$ - m

DETERMINATION OF WATER SOLUBLE ASH:

APPARATUS REQURIED : Muffle furnance, Silica Crucible with cover, Spatula, Desiccator, analytical Balance.

PROCEDURE : Boil the ash for 5 minutes with 25ml. of water, collect insoluble matter in a silica crucible, and on an ash less filter paper, wash with hot water and ignite to constant weight. Subtract the weight of insolule matter from the weight of ash the difference in weight represents the water soluble ash. Calculate the percentage of water soluble ash with reference to the air dried drug.

FORMULA : Water soluble ash =(m_2-m_1) x $100/m_1$ - m

DETERMINATION OF ALCOHOL SOLUBLE EXTRACTIVE :

APPARATUS REQUIRED: Conical flask (100/250 ml), Mechanical Shaker, Evaporating Dishes, Filter paper, Funnels. Analytical balance.

PROCEDURE: Macerate 5 g. of the air dried drug, coarsely powdered, with 100 ml of alcohol of the specified strength in a closed flask for twenty-four hours. Filter rapidly takng precautions against loss of water. Evaporate 25ml. of the filtrate to dryness in a tarred flat bottom shallow dish and dry at 105^{o}C to constant weight and weight. Calculate the percentage of water soluble extractive with reference to the air dried drug.

FORMULA : Solubility = Average x 500

DETERMINATION OF MOISTURE CONTENT (LOSS ON DRYING):

APPARATUS REQURIED: Hot air Oven, Evaporating Dishes.

PROCEDURE: Take 10g of drug after accurately weighing in a tiered evaporating dish. After placing the above said amount of the drug in the tiered evaporating dish dry at 105^{o}C for 5 hours and weight. Continue the drying and weighing at one hour interval until difference between two successive weighings corresponds to not more than 0.25 percent. Constant weight is reached when two consecutive weighing after drying for 30 minutes and cooling for 30 minutes in a desiccator, show not more than 0.01 g. difference.

FORMULA - AVERAGE X 100 / sample

DETERMINATION OF pH VALUE: The pH value of an aqueous liquid may be defined as the common logarithm of the reciprocal of the hydrogen ion concentration expressed in g per liter.

METHOD: Operated the pH meter and electrode system according to the manufacturer's instructions. Standardise the meter and electrodes with 0.5 M potassium hydrogen phthalate (pH 4.00) when measuring an acid solution, or with 0.05M sodium borate when measuring an alkaline solution.

At the end of a set of measurement, take a reading of the solution used to standardize the meter and electrode. This reading should not differ by more than 0.02 from the original value of which the apparatus was standardized.

PHYTOCHEMICAL TESTS FOR ACTIVE CONSTITUENTS (total alkaloids/total tannin/resins etc.)

Alkaloid : Alkaloids are basic nitrogenous compounds with definite physiological and pharmacological activity. They contain at least one nitrogen atom in a ring. The alkaloids are colourless or white crystalline mass, insoluble in water but soluble in organic solvents.

Tests for alkaloids:

Wagner's Test :1 ml of alcoholic extract of the drug with 1.5% v/v HCL acid and add few drops of Wagner's reagent; a yellow brown ppt is formed.

Mayer's test : Add few drops of Mayer's reagent to 1 ml of aqueous extract of the drug a white yellowish ppt is produced.

Tannins : They are non nitrogenous complex compounds, commonly found dissolved in the cell sap. They are the derivatives of phenol and usually related to glycosides.

Tests for tannins: To 1-2 ml extract of the drug add few drops of 5% $FeCl_3$ solution a green colour indicate the presence of tannin.

Resin : Resins are solid or semisolid exudates of plants. Chemically they are complex mixtures of compounds like aliphatic, alcohols, resin acids; phenols and terpenes. They are practically insoluble in water but dissolve in organic solvents.

Test for resin : Dissolve 1 ml of aqueous extract of the drug in acetone and pour the solution into distilled water turbidity indicates the presence of resin.

Protein : They are made of basic unit of amino acid which are linked together in chain or rings through peptide bonds. Carboxylic group of one amino acid, is joined with amine group of another amino acid.

Tests for Protein:

Biuret Test: 1 ml of hot aqueous extract of the drug add 1 ml of 4% NAOH solution followed by 1-2 drops of 1% copper sulphate solution a red or violet colour was obtained.

Millon's Test: Dissolve small quantity of aqueous extract of the drug in 1 ml of distilled water and add 5-6 drops of millon's reagent a white ppt is formed which turned red on heating.

Carbohydrate: Those molecules which contain at least one ketone and one aldehyde group at second carbon atom in chain.

Fehling's Test: 2 ml of aqueous extract of the drug add 1 ml of a mix of equal part of Fehling solution A and Fehling solution B and boil the content for few minutes a red or brick red colour is obtained..

Molish's Test: In a test tube containing 2 ml of aqueous extract of drug add drops of Molish's reagent. To this solution add 1 ml of concentrated sulphuric acid carefully from the side of the test tube, so that the acid formed a layer beneath the aqueous solution without mixing in it. Appearance of a red violet ring at the common surface of the liquids indicates the presence of sugars on mixing whole solution becomes violet red with blue ppt.

Flavonoids: Flavonoids contains fifteen carbon atom in their basic nucleus and these are arranged in a C_6-C_3-C_6 configuration.

Test for Flavonoids:

Shinoda Test : In a test tube containing 0.5ml of alcoholic extract of the drug add 5-10 drops of dilute HCL acid followed by a small piece of Mg. if the presence of Flavonoids, a pink reddish brown or brownish colour obtained.

Saponin : Saponin are highly complex glycosides which are widely distributed in higher plants.

Test for Saponin:

Forthing Test : In a test tube containing about 5ml of an aqueous extract of the drug add a drop of sodium bicarbonate(NaHCO3) shake the mixture vigoursly. Honey comb like Forthing formed which persisted for more than 30 minutes.

Steroids : Steroids are tetra cyclic compounds characterized by the presence the presence of perhydro(1, 2)cyclo pentenophenanthrene ring system. The three cyclohexane rings are designated as A,B,C and the cyclopentane ring is the D ring. They are colourless crystalline compounds widely distributed in plants and animals. Stigmasterol, testosterone, progesterone and hydrocortisone are the important steroids.

Test for steroids:

Liebermann Burchards Test : To a chloroform solution of the drug, few drops of acetic anhydride and 1 ml of concentrated sulphuric acid are added. Blue or blood red colour is produced.

Salkowaski Test : Add 1 ml of concentrated H_2SO_4 to 2 ml of chloroform extract of the drug carefully from the side of the test tube. A red colour is obtain on the chloroform layer.

Starch: Starch are polysaccharides which are made from linked many pyranose structure of glucose, which are attached with α,1,4 glycosidic linkage.

Test for starch: Dissolve 0.015g of lodine and 0.075g of Potassium lodide in 5ml distill water and add 2-3 ml. of an aqueous extract of the drug. A blue colour indicates the presence of Starch.

HPTLC FINGERPRINTING: High performance thin layer chromatography is a sophisticated and automated form of TLC technique. The method is used for separation of the components present in the mixture both qualitatively as well as quantitatively.

PROCEDURE :

a. Weigh 5 g of sample.
b. Add 100 ml distilled ethyl alcohol.
c. Keep it over night with occasional shakings.
d. Boil for 5-10 minutes on a water bath.
e. Cool and filter.
f. Concentrate the filtrate and make up to 10ml in a volumetric flask.
g. Switch on the HPTLC applicator.
h. Regulate the pressure of the nitrogen gas to 3.5kg. in the regulator, wash syringe with test solution.
i. Fill the syringe with extract prepared above for the qualitative analysis.
j. Program the applicator as directed.

PARAMETERS INVOLVED IN PROGRAMMING ARE :

i. Plate width (used maximum 20 cm)
ii. Start position (generally 10 mm)
iii. Band width (generally 10 mm)
iv. Space (generally 10mm)
v. Syringe of 10 microlitres are in use. For crude plant extract syringes of 100 microlitres are in use.
vi. Speed (10 microlitres/sec)

RF Value : Measure and record the distance of each spot from the point of its application and calculate the RF value by dividing the distance travelled by the spot and distance travelled by the font of mobile phase.

MICROBIAL LIMIT TESTS : The following tests are designed for the estimation of the number of viable aerobic micro-organism present and for detecting the presence of designated microbial species in pharmaceutical substance.

Total Aerobic Microbial Count :

Apparatus required : Petri plates. Autoclave, Laminar Air Flow, Colony counter Media.

For bacteria : Using Petri dishes 9 to 10 cm in diameter, add to each dish a mixture of 1 ml of the pretreated preparation (Inoculum) and about 15 ml of liquefied casein soybean digest agar. Alternatively, spread the pretreated preparation on the surface of the solidified medium in a petri dish of the same diameter. Prepare at least two such Petri dishes using the same dilution and incubate at 30^o-35^o for 5 days, unless a more reliable count is obtained in a shorter time. Count the number of colonies that are formed. Calculate the results using plates with the greatest number of colonies but taking 300 colonies per plate as the maximum consistent with good evaluation.

For fungi : Using Petridishes 9 to 10 cm in diameter, add to each dish a mixture of 1 ml of the pretreated preparation (Inoculum) and about 15 ml. of liquefied dextrose agar in place of soybean casein digest agar and incubate in a shorter time. Calculate the results using plates with not more than 100 colonies.

Tests for Specified Micro-Organisms :

Apparatus required : Petri plates, Autoclave, Laminar Air Flow, Colony counter Test tubes. The medicinal drugs are tested whether the pathogenic bacteria are present in it or not and different media were used for the isolation this bacteria. These pathogenic bacteria are :

i. *Salmonella*
ii. *Pseudomonas* sp.
iii. *Staphylococcus*
iv. *E. coli.*

Procedure for testing microbial limit test :

Total Bacterial count: 1 ml of the pretreated inoculum was taken and about 15ml of liquefied casein soyabean digest agar was poured in petri dish in duplicate and incubate at 35+ -2°C for 48 hours, unless a more reliable count was obtained in shorter time. The number of colonies observed were calculated with the help of digital colony counter.

Test for yeast & mould : 1 ml of the pretreated inoculum was taken and about 15 ml of liquefied potato dextrose agar was poured in duplicate. After gentle rotation & solidification of agar, the plates were incubated at 22°C to 25°C for 5 days, unless a more reliable count was obtained in shorter times. Then result using plates with not more than 100 colonies were calculated.

Test for specified micro-organism:

Test for E.coli : 1 gm of sample taken in 100 ml of sterilized nutrient Broth in a sterilize screw-capped container, shaken and allowed to stand for 1 hour and shaken again. I Loosen the cap incubate at 37°C for 18-24 hours.

Primary Test: 1 ml enriched culture was inoculated into a tube containing 5 ml of Macconkey broth incubated in a water bath at 37°C for 48 hours observation was noted. A small portion of culture was streak on the surface of vogel-johnson agar each plated on petridishes in triplicate covered and inverted the plates,incubated at 37°C for 18-24 hrs.

Secondary Test: By means of a inoculating loop, a small portion from the enrichment culture was streaked on the surface of Macconkey medium. Covered & inverted the plates and incubated at 35+ -2°C for 24 hours upon examination, if none of the colonies are brick red in colour and have a surrounding zone of precipited bile the sample meets the requirement of the test for the absence of the E.coli.

Test for salmonella: 1 gm of sample taken in 100ml. Nutrient Broth in a sterile screw capped container, shaken and allowed to stand for 1 hour and shaken again. Loosen the cap & incubated at 37°C for 24 hours.

primary test: 1ml enriched culture was inoculated to each of the two tubes containing (a) 10ml of *selenite* F broth and (b)tetra thionate-bile brilliant green broth and incubate at 37◦C for 48 hrs.

Secondary test: Subculture from each of these two culture a small portion of inoculums on brilliant green agar and xylose lysine-deoxycholate agar.cover and invert the plates and incubate at 37°C for 18-24 hrs.

Test for pseudomonas aeruginosa: 1 gm of sample taken in 100ml of sterilized soyabean casein digest broth in a sterile screw capped container, mixed and incubated at 37°C for 24-48 hours. A small portion of culture was streak on the surface of Cetramide agar medium each plates on Petridish. Covered and inverted the plates and incubated at 37°C for 18-24 hours. Upon examination if none of the plates contain colonies having greenish fluorescence growth in UV light, the sample meets the requirement for the freedom from Pseudomonas aeruginosa.

Test for staphylococcus aureus: 1 gm of sample taken in 100 ml of sterilized soybean casein digest broth in a sterile screw capped container, mixed and incubated at 37°C for 24-48 hours. A small portion of culture was streak on the surface of Cetramide agar medium each plates on Petridish . Covered and inverted the plates and incubated at 37°C for 18-24 hours. Upon examination, if none of the plates contain no growth having black colonies surrounded by yellow zone, the sample meets the requirement for the freedom from staphylococcus aureus.

RESULTS

Physicochemicalanalysis – *Butea monosperma*

(i) Total Ash

S.No.	Preweight of Crucible	Postweight of crucible	1st Day	2nd Day	3rd Day	Difference	Standard Value
1.	39.4236	41.4247	39.8801	39.8545	39.8505	0.4269	> 12%
2.	37.3246	39.3263	37.7715	37.7538	37.7500	0.4254	
3.	39.6117	41.6112	40.0597	40.0411	40.0380	0.4263	
4.	35.5705	37.5703	36.0135	35.9986	35.9946	0.4241	
5.	37.0470	39.0475	37.4906	37.4776	37.4745	0.4275	
6.	34.4149	36.4153	34.8543	34.8441	34.8413	0.4264	

Average = 0.4261; Formula : Average Value x 100/2

= 0.4261 x 10012

= 21.30%

(ii) Acid insoluble Ash : *B. monosperma*

S.No.	Preweight of Crucible	Postweight of crucible	1st day	2nd day	3rd day	Standard Value
1.	39.4233	40.4800	39.4602	39.4598	39.4600	>15%
2.	37.3243	38.2570	37.3487	37.3481	37.3435	
3.	39.6120	40.6446	39.6533	39.6526	39.6548	

Formula : Average Total ash weight - Average total Post weight
= 39.7938 - 38.8211
= 0.9727
Total Acid Insoluble = Average x 100/2
= 0.9727 x 100/2
= 48.6%

(iii) Water Soluble Ash : *B. monosperma*

S.No.	Preweight of Crucible	Postweight of crucible	1st day	2nd day	3rd day	Standard Value
1.	35.5709	36.9622	35.9538	35.9525	35.9526	Not Avail in API
2.	37.0471	38.3614	37.4316	37.4301	37.4299	
3.	34.4164	35.7793	34.8070	34.8057	34.8054	

Formula : Average Total ash weight - Average Total Postweight
= 37.0343 - 36.0626
= 0.9717
Total water soluble = Average x 100/2
= 0.9717 x 100/2
= 48.5%

(B) Solubility :

(a) Alcohal soluble extractive (ASE)

S.No.	Preweight of Petridish h	Post weight of Petridish h	Difference	Average Difference	Standard Value
1.	32.0727	32.0805	0.0078	0.074	<10%
2.	26.1029	26.1102	0.0073		
3.	32.8235	32.8306	0.0071		

Formula = Average x 500
= 0.0074 x 500
= 3.7%

(b) Water soluble extractive : (WSE)

S.No.	Preweight of Petridish h	Post weight of Petridish h	Difference	Average Difference	Standard Value
1.	32.3146	32.3501	0.0355	0.0348	<4%
2.	29.0013	29.0358	0.0345		
3.	34.1736	34.2065	0.0330		

Formula = Average x 500
= 0.0348 x 500
= 17.41%

(C) Loss on Drying : *B. monosperma*

S.No.	Preweight of Petridish h	Postweight of Petridish h	Post weight I	Post weight II	Difference	Average Difference
1.	22.0733	24.0338	23.9213	23.9230	0.1108	0.1092
2.	22.8493	24.8486	24.7360	24.7375	0.1111	
3.	22.2394	24.2388	24.1314	24.1331	0.1057	

Formula = Average x 100/Sample
= 0.1092 x 100/2
= 5.4%

(D) P_H Value - *B. monosperma*

= 5.40 Acidic

Phytochemical Test : (**A**) *B. monosperma*

(**B**) *I. tinctoria*

S.No.	Experiments	Standard Results	A	B
1.	Alkaoid	Yellow/brown ppt is obtain	-nt	-nt
2.	Flavonoid	Pink, reddish or brown colour is formed	-nt	-nt
3.	Resin	Turbidity formed	+nt	-nt
4.	Saponin	Honey comb like formed	-nt	-nt
5.	Carbohydrate	brick red formed	-nt	+nt
6.	Tanin	Brown, green colour formed	+nt	-nt
7.	Protein	Red/Voilet is obtain	-nt	-nt

PHYSICOCHEMICAL ANALYSIS -- *Indigofera tinctoria*

(i) Total Ash

S.No.	Preweight of Crucible	Postweight of Crucible	Ist day	2nd Day	3rd Day	Diffferent
1.	34.0640	36.0667	34.1410	34.1400	34.1399	0.0759
2.	34.0452	36.0486	34.1295	34.1290	34.1286	0.0834
3.	37.5602	39.5647	37.6495	37.6489	37.6494	0.0892
4.	34.5347	36.5374	34.6188	34.6186	34.6186	0.0839
5.	36.7261	38.7289	36.8120	36.8115	36.8115	0.0854
6.	35.3220	37.3240	35.4096	35.4095	35.4095	0.0875

Average = 0.0842

Formula = Average Value x 100/2

= 0.0842 x 100/2

= 4.21%

(ii) Acid Insoluble Ash :

S.No.	Preweight of Crucible	Postweight of Crucible	1st day	2nd day	3rd day	Standard Value
1.	34.9058	35.9022	34.9284	34.9287	34.8797	<0.7%
2.	33.6348	34.6254	33.6580	33.6584	33.6123	Acco to
3.	37.1320	38.1555	37.1582	37.1585	37.1084	API.

Formula = Average total ash weight - Average Total Post weight

= 36.2277 - 35.2001 =1.0276

Total acid Insoluble = Average x 100/2

= 1.0276 x 100/2

= 51.38%

(iii) Water Soluble Ash :

S.No.	Preweight of Crucible	Postweight of Crucible	1st day	2nd day	3rd day	Standard Value
1.	34.5370	35.5650	34.5815	34.5815	34.5329	>4.0%
2.	36.7292	37.7864	36.7992	36.7996	36.7484	Acco.to
3.	35.3241	36.3533	35.3876	35.3873	35.3378	API

Formula = Average total ash weight - Average Total Post weight

= 36.5682 - 35.53

= 1.03

Total water soluble = Average x 100/2

= 1.03 x 100/2

= 51.911%

(B) Solubility :

(a) Alcohal soluble extractive (ASE)

S.No.	Initial weight of Petridish h	Final weight of Petridish h	Difference	Average Difference	Standard Value
1.	34.1708	34.1792	0.0084	0.0083	>3.0% Acco to API.
2.	32.8202	32.8296	0.0094		
3.	33.1760	33.1833	0.0073		

Formula = Average x 500 = 4.18%

(b) Water Soluble extractive (WSE)

S.No.	Initial weight of Petridish h	Final weight of Petridish h	Difference	Average Difference	Standard Value
1.	36.0950	36.1048	0.0028	0.0082	>4.0% Acco to API.
2.	45.7490	45.7563	0.0078		
3.	42.6524	42.6601	0.0077		

Formula = Average x 500 = 4.13%

(C) Loss on Drying -

S.No.	Initial weight of Petridish h	Weight of Petridish h with Sample	Post weight I	Post Weight II	Difference	Average Difference
1.	22.3984	24.4008	24.3131	24.3123	0.0885	0.0899
2.	22.2893	24.2896	24.1962	24.1977	0.0919	
3.	22.1411	24.1410	24.0499	24.0505	0.0895	

Formula = Average x 100/Sample
= 0.0899 x 100/2
= 4.49%

(D) P_H Value - 5.2 Acidic

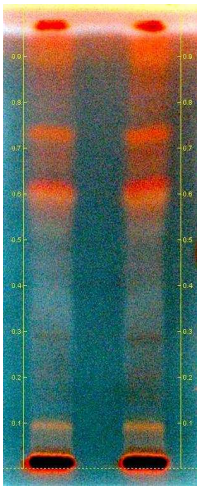
HPTLC of *Butea monosperma* :

Family - Leguminacea

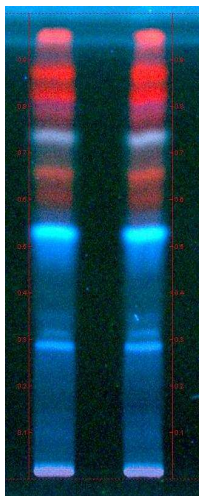
Part Used - Stem Bark

Chemical Constitute - Resin, tannin

HPTLC Finger Printing of test solution of Butea Monosperma After and before derivatization:-



366nmAD



366nmBD

R.F. Value: **Before Spray 366mm.**

Rf. Value	Colour	A	B
1.	Blue	0.29	0.29
2.	Blue	0.31	0.31
3.	Fluorescent	0.53	0.53
4.	Red	0.66	0.66
5.	Pink	0.73	0.73
6.	Red	0.80	0.80
7.	Dark Red	0.83	0.83
8.	Pink	0.87	0.87

After Spray 366mm

Rf. Value	Colour	A	B
1.	Brick red	0.10	0.10
2.	Red	0.29	0.29
3.	Brick red	0.59	0.59
4.	Brick Red	0.73	0.73
5.	Brick red	0.91	0.91
6.	Red	0.97	0.97

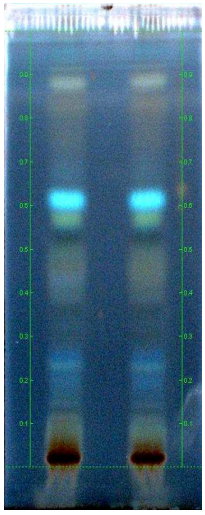
HPTLCof NILI:

Family - Leguminaceae

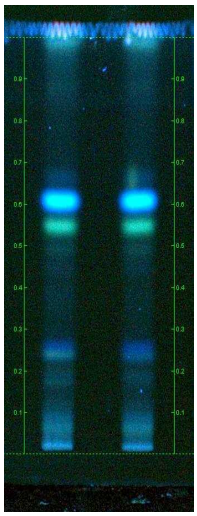
Part Used - Root

Chemical Constitute - Carbohydrate (INDICAN)

HPTLC fingerprinting of test solution of Indigofera tinctoria After and Before derivatization:-



366 nm AD



366 nm BD

RFValue:

366mm Before spray :

Rf.Value	Colour	A	B
1.	Light green	0.07	0.07
2.	Blue	0.10	0.10
3.	Blue	0.25	0.25
4.	Green	0.55	0.55
5.	Fluorescent	0.61	0.61
6.	Green	0.99	0.99

366mm After Spray :

Rf. Value	Colour	A	B
1.	Brown	0.08	0.08
2.	Light Blue	0.23	0.23
3.	Blue	0.55	0.55
4.	Brown	0.56	0.56
5.	Fluroscent	0.61	0.61
6.	Brown	0.88	0.88

Microscopic Characters of Palas Stem Bark :

Mature Bark shown rhytidoma consisting of altering layers of cork secondary cortex and phloem tissue, cork cells thin walled, 5-10 or more layerd, rectangular, dark brown, secondary cortical cells, often in groups, having brown colour sometimes containing mucilage and other materials found scattered in this zone beneath this zone regular cork consisting of 4-12 rows of radially arranged, rectangular cells followed by a zone of 2-4 layers of sclerieds, secondary phloem consisting of sieve tube, companion cells phloem parenchyma, phloem fibres, crystal fibres, transversed by phloem rays in outer and middle phloem regions phloem tissues get crushed and form tangential bands of ceratenchyma, phloem fibres arranged in tangential bands alternating with sieve tubes and phloem parenchyma, most of fibre groups contain Prismatic crystal of calcium oxalate formign crystal sheath, in macerated Preparation phloem fibres appear thick walled, lignified elongated with tapering of bifurcated ends, crystal fibres divided into a number of chambers containing a prismatic crystal of calcium oxalate in each chamber phloem rays multiseriate 4-12 cells wide, 7-50 cells in height, straight, prismatic crystals of calcium exalale found scattered in the secondary phloem tissues and phloem rays, starch grains simple or compound having 2-3 components measuring 2.75-13.75u in diametre found scattered in Phloem parenchyma and phloem ray cells abundantly, tanniferous cells and sec. canities also occur in sec. phloem.

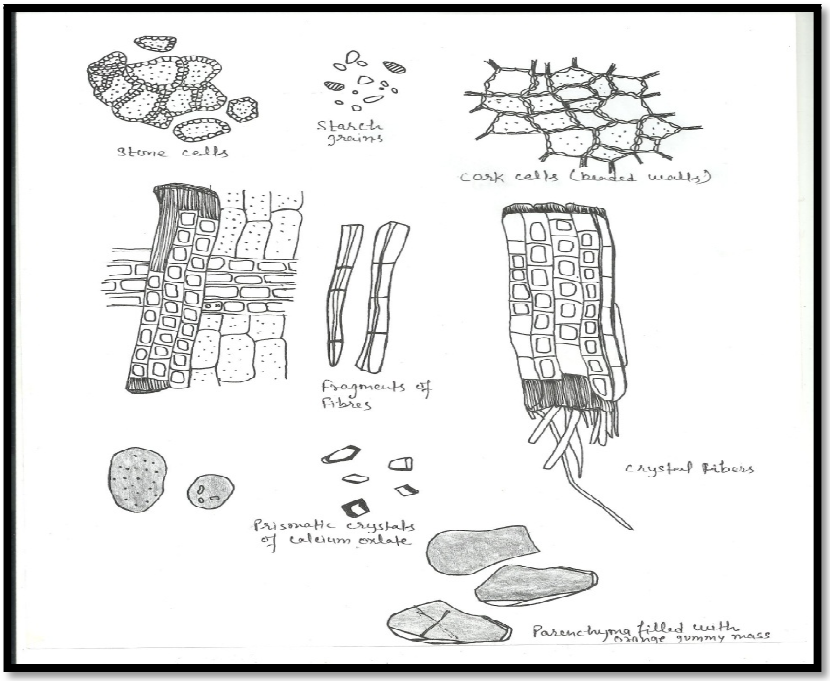


Fig. 1: Microscopic structure of palash (stem bark)

Powder microscopy observation of Butea monosperma:

Dark brown, shows cork cells with beaded walls, fragments of fibers, starch grain, stone cells, crystals fibers, part of phloem in radial longitudinal section, crystals of calcium oxlate, parenchyma filled with orange gummy mass.

Microscopic characters of Nili Root :

Shows a narraow zone of cork consisting of 4-10 layers of tangentially elongated rectangular to Polygonal, thin walled cells, group of fibre.

Measuring 11-17 u in dia, thick walled and lignified with wide lumen, secondary phloem composed of usual elements, wood occupies bulk parts of the root, consisting of usual elements, vessels solitary or 2-4 groups having simple pits, fibres present in the form of alternating to polygonal in shape and attached on both the opposite sides of vessels meddulary rays 1-4 cells wide, Prismatic crystals of calcium oxalate Presnet tin secondary cortex, Phloem and xylem parenchyma rays, oil globlues presnet in cortex and phloem Parenchyma, starch grains simple, round to oval, measuring 3-11u in dia, present in the cortex phloem Xylem parenchyma and rays.

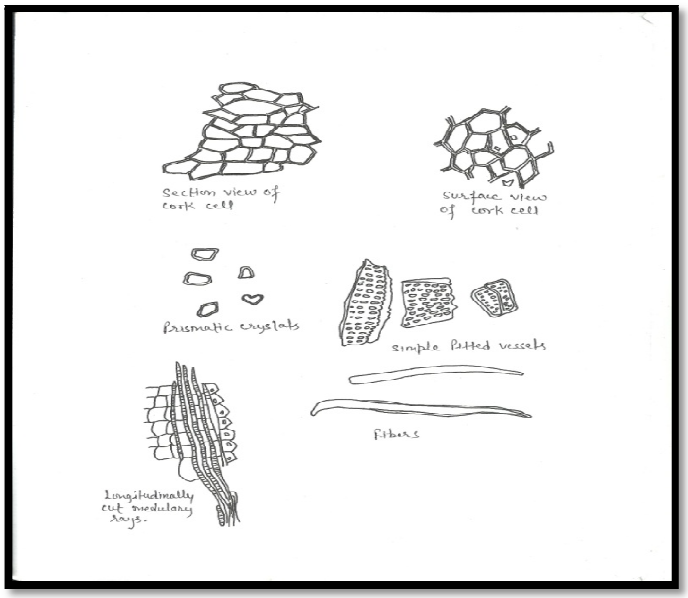


Fig. 2: Microscopic structures of nili Root

Powder microscopy observation of indigofera tinctoria:

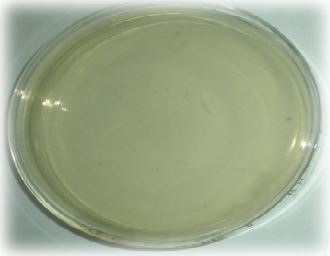
Surface view of cork cell, simple pitted vessels, prismatic crystals, fibers, longitudinally cut medullary rays.

Test for Microbial Contamination :

The following test was carried out and the results were given the below table :

- Determination of Total Bacterial count
- Determination of yeast & mould
- Enumeration of *E.coli*
- Enumeration of *Salmonella* sp.
- Enumeration of *Pseudomonas aeruginosa*
- Enumeration of *Staphylococcus aureus*.

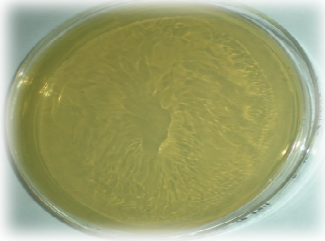
(A) Observation : Total Bacterial count of Butea Monosperma



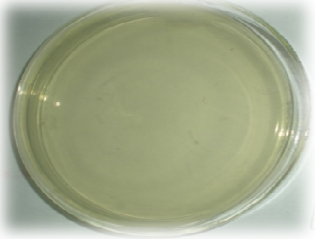
Control (TBC)

Test Culture (+nt)

(B). Observation : Total Bacterial count of Nili

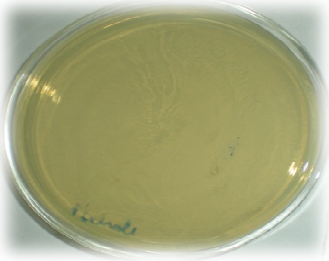


Control (TBC)



Test Culture (-nt)

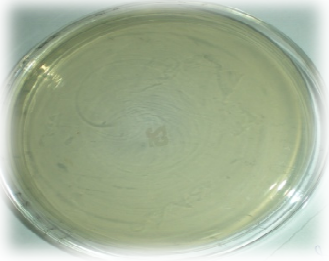
(A) Observation : Yeast & Mould of Butea Monosperma



Control Y & M

Test culture(+nt)

(B) Observation of Yeast &mould of Nili

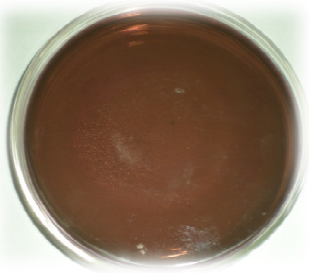


Control Y & M

Test culture(-nt)

(A) Enumeration of *E. coli* in *Butea monosperma*

Control (*E.Coli*)



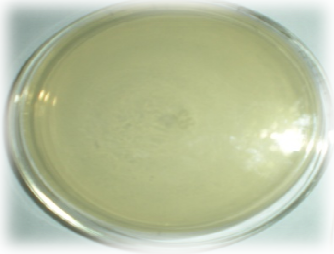
Test Culture(Absent)

(B) Enumeration of *E. coli* in Nili

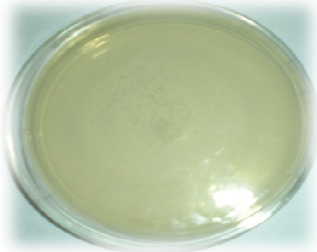
Control (*E.Coli)*

Test Culture (Absent)

(A) Enumeration of salmonella in *Butea monosperma*

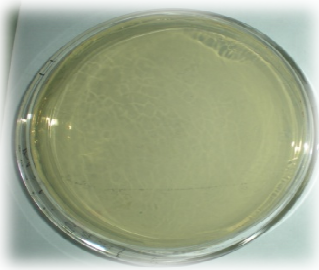


Control (salmonella)

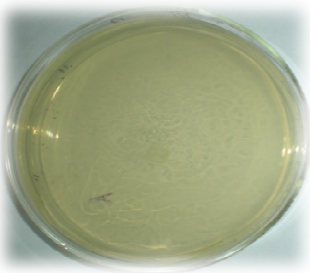


Test Culture (-nt)

(B) Enumeration of salmonella in Nili



Control (salmonella)



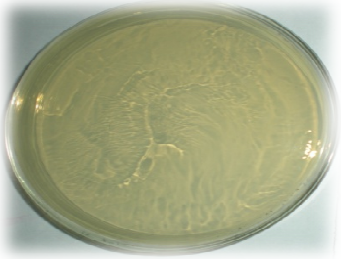
Test Culture (-nt)

(A) Enumeration of pseudomonas aeruginosa in *Butea monosperma*

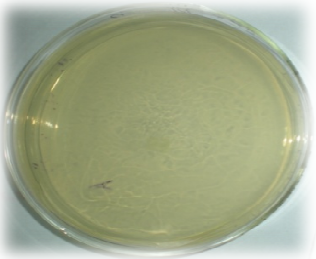
Control (pseudomonas)

Test Culture (-nt)

(B) Enumeration of pseudomonas aeruginosa in Nili

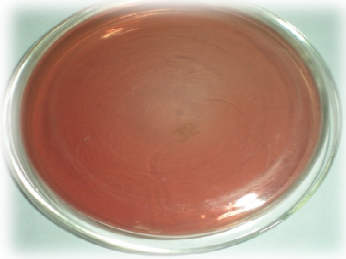


Control (pseudomonas)



TestCulture(-nt)

(A) Enumeration of Staphylococcus aureus in *Butea monosperma*

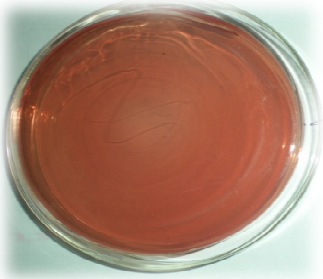


Control (S.aureus)

Test Culture (-nt)

(B) Enumeration of Staphylococcus aureus in Nili

Control (*S. aureus*)



Test Culture (-nt)

DISSCUSION

Pharmacogonostical evaluation of Butea monosperma and Indigofera Tinctoria was carried out as per standard procedure. During the microscopy it has been found that work cells (beaded walls) starch grains, stone cells, crystal fibres, Prismatic crystals of calcium oxlate, Parenchyma filled with orange gummy mass, simple pitted vessels with longitudinally cut medullary rays.

Physicochemical and phytochemical study of Butea monosperma and Indigofera Tinctoria were done and found that the some results were variated from standard value which is given in API, due to variation of atmospheric character & ecological phenonmenon, from there the sample of plant was took at diferrent soil profile region,

Chemical constituents Resin,Tannin, in Bark of B. Monosperma & carbohydrate or glycoside (Indican), were +nt in root of Indigofera Tinctoria as phytochemical standard test,

HPTLC Profiles of B. Monosperma & Indigofera Tinctoria were determines. During the microbial contamination it was found that the sample(bark,root) having permissible microbial lode according place where tree/Plant adapts in soil profile and it can be use for further study.

CONCLUSION

- The present research work reveals that these medicinal herbs were scientifically validated with special attention on their standardization as per ISM and as the result shows all the quality control parameters were tested like Quantitative determination, Descriptive identification, Determination of Physico-chemical properties, Phytochemical analysis, HPTLC assessment, Microbiological assesment and all results were found to comply with API standards.
- Physicochemical analysis were useful for proceeding complete research determinations.
- Microscopy photographs of herbel drug powder are useful to determine anatomical features of cells and tissues in drug sample.
- The various phytochemicals shall be identified by phytochemical tests and HPTLC assessment on extractive and used as bio marker for medicinal plants.
- Microbiological assisment are also helpful to determine various microbes in drug sample. A systematic pharmacognstic study for two medicinal drugs that is *B. monosperma* and *I. tinctoria*.
- Since,known a day consumer are moving towards ayurvedic treatment rather than allopathic because of their less side effects.
- We are taken as a interest. It was observed that these drugs have been although traditionally have been used for fighting against several chronic disease.
- Our studied drugs were found to be satisfactory from Standardization and quality control point of view and can be confidently been used as a medicine for human consumption.

Acknowledgement

First of all, I would like to express my profound thanks to respected**, DR. MANOJ TRIPATHI (SRO) Mr. SHARDA PRASAD TRIPATHI, Mr. ASHOK TIWARI, Mr. PUSHPENDRA SHUKLA,** for giving me warm encouragement and Inspiration for my task. His precious guidance cannot expressed only with thanks.

I feel great pleasure to expression my profund thanks to My Principal **Dr.H.D GUPTA(HOD)** Department of Bio-chemistry Govt. T.R.S college Rewa(M.P)for his well valuable guidance through tenure of work of project.

I am also thankful to my parents **DR. R.K SONI** to supported to me and constant encouragement & my friends which are worked with me during research time.

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7. Microbial assisment, HPTLC is according to Protocol of DRI (Chitrakoot).