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Pharmacognostic evaluation of Laata- A traditional, nutritious, dietary formulation, prevalent in the Chitrakoot region of Madhya Pradesh, India

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Chitrakoot region is a remote area situated in the northern region of Satna district of Madhya Pradesh and the southern region of Chitrakoot district of Uttar Pradesh, India. It has strong local health traditions in which medicinal plants play an important role. The region has well-developed oral medical includingnutritional wisdom in the form of tribal medicines, home remedies, local health traditions,and nutritional food materials. There are several folklore delicacies with herbal compound formulations used to maintain a healthy life. The present study highlights the documentation of folklore practices and their standardization with special reference to Laata (a traditional herbal nutritious compound formulation) inthe Chitrakoot region of Madhya Pradesh. For pharmacognostic investigation macroscopy, powder microscopy, physicochemical parameters, detection of heavy metals, nutritional value analysis, pesticide residue tests, screening of microbiological parameters, and high-performance thin layer chromatography (HPTLC) fingerprints profile of methanolic extract were performed. Microbiological analysis of pathogenic bacteria, *viz.Salmonella* sp., *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were done and found absent in Laata samples, similarly, detection of heavy metals (Pb, Cd, As & Hg), and 42 types of pesticides residue tests were performed and found under limits/absent as per WHO guidelines. Nutritional value tests of four compound formulations of Laata were analyzed such as total calories range 395.10-469.91kcal/100gm, total carbohydrates range45.60-57.18g/100gm, total fat 17.40-22.63g/100gm, protein 10.75-13.56g/gm, vitamin c 2.00-3.39mg/100gm, dietary fibre 3.90-4.89g/100gm, Iron8.60-10.48mg/gm, Calcium 12.50-17.72mg/gm, Sodium 62.34-80.20mg/gm and Potassium 105.60-155.70mg/gmwere found.These findings are indications thatLaata (a traditional herbal nutritious compound formulation) is a very good traditional herbal recipe used by the tribal of Chitrakoot region to maintain good health. These practices if integrated with the modern healthcare system could elevate the health status of thousands of rural people as well as urban people. This study may also help in the preparation of Laata and their quality evaluation.

Keywords: HPTLC fingerprinting, Laata, Microbiology,Nutritional value, Standardization.

Introduction

India is endowed with one of the richest expertise in spiritual, religious, cultural, traditional food materials, and folk-lore medicines. As per our own experience and field survey, we can frequently say that veryless traditional systems have a fair level of documentation in the Chitrakoot region. Most of the little traditional system of knowledgeis transmitted orally from generation to generation. India has a vast store of oral medical wisdom available in the form of tribal medicines, home remedies, and local health traditions[1-3]. The tribal communities, villagers living in remote areas, who are untouched by modern civilization use plants for their basic health care needs. Chitrakoot is one such area situated in the northern region of Satna District of Madhya Pradesh, India.It lies between $80^0$52’ to 80^0 73’ in latitude and $25^0$10’ to $25^0$52’ longitude, covering an area of 1584sq km. surrounded by lush green hills of the legendary Vindhyachal range and inhabited by

various tribal communities like Mawasi, Khairwar, Kol, Gond, etc. Depending on the availability of plants in this area, these people have developed herbal remedies and nutritious herbal compound formulations which they use to attain good health[4].There are a lot of traditional societies in rural areas of Chitrakoot region that have rich health traditions and traditional health practitioners who are propagators of these traditions make use of medicinal plants in various practices and traditional recipes.[5]The young generation due to urbanization and fast-changing trends in their lifestyle does not want to follow in the footsteps of their forefathers. As a consequence, this knowledge is getting eroded. These rich traditions of India that continue to provide the health care needs of the vast majority of rural masses need to be protected before it is lost forever[6].In this concern,there is an urgent need for documentation of folklore practices and their standardization.It is observed that the tribal of the Chitrakoot region use nutritious herbal compound formulations oftheir own prepared that is called Laata(a traditional herbal nutritious compound formulation) in the local language. Ruralpeopleeat Laataasa breakfast which is prepared from mainly "Mahua flower" with few other ingredients. *Laata*is a complete nutritional recipe, with rich contents of essential nutrients. Despite the numerous uses of folklore knowledge the work has been undertaken to document folklore practices and their pharmacognostic study with special reference to Laata (a traditional herbal nutritious compound formulation) inthe Chitrakoot region of Madhya Pradesh. For standardization of Laata,the work deals with documentation of herbal, and compound formulations of Laata, selection of ingredients and their morphological, anatomical evaluation, physicochemical tests, preliminary phytochemical screening, heavy metals test, nutritional value analysis, pesticides residue tests, microbiological screening, and High-Performance Thin Layer Chromatography.

Methodology

Systematic surveyand documentation of folk-lore/traditional knowledge

For the present study a systematic survey and documentation of traditional knowledge[7] with special reference to Laata (a nutritious herbal compound formulation) used by villagers in the Chitrakoot region of 24 tribal (Kol, Gond, Mawasi &Khairwar) dominant villages surveyed, *viz.* Umariha, Mudkhoha, Chauraha, Kelhoura, Dalela, Kanpur, Amiliya, Barua, Bhargawan, Bundelapur, Chandai, Devlaha, Tagi, Hiraundi, Kailashpur, Kathauta, Koldari, Parewa, Patna, Patni, Pindra, Piparitola, Raiya and Turra. These villages are located in a radius of 50 km around Chitrakoot town. A total of 760 interviews conducted in 98 intensive field visits were carried out during 2019-2020, covering almost all the seasons of the year. The 45-80-year-old traditional healers, local vaidyas, and especially experienced women who have been actively engaged in the preparation of Laata(a traditional recipe)as well as ethno-medicinal practices were interviewed and their prior informed consent was recorded. Detailed information regarding the preparation and uses of Laatasuch as plant (ingredients) name, parts used, ratio of ingredients, mode of preparation, mode of application, doses and duration, benefits and age groups, etc. has been recorded with the help of standard questionnaires. The information was crosschecked on different field visits to the same localities or in the other localities. The voucher specimen was also collected, identified with the help of local floras, prepared the herbarium, and preserved at the research laboratory, ArogyadhamCampus, Deendayal Research Institute, Chitrakoot, dist. Satna (M.P.). The generated data were entered in

Microsoft World separate sheets. It was checked and edited for error and coded (Fig. 1).

Collection of samples and formulation preparation

The fresh ingredients of Laata compound formulations were collected from forest Mahua (*Madhuca longifolia* (Koen.), family-Sapotaceae) flowers, Chirounji(*Buchananialanzan*Spreng., family-Anacardiaceae) seeds, Gond,exude(plants), Ashwagandha*(Withaniasomnifera*(Linn.) Dunal, family-Solanaceae) root, Safed Musali (*Chlorophytum borivlianum (Roxb.),*family-Liliaceae*)* rootand from villagesTil*(Sesamum indicum* Linn., family-Pedaliaceae) seeds, Alasi (*Linum usitatissimum*Linn.,family-Linaceae) seeds and also purchased from Chitrakoot market Dist. Satna, Madhya PradeshKaju (*Anacardium occidentale* Linn., family-Anacardiaceae) fruits,Badam (*Prunus dulcis* Linn., family-Rosaceae) fruits. Samples were identified and authenticated as per standard procedure[5]by Dr. Manoj Tripathi Senior Scientist, Deendayal Research Institute Chitrakoot. Fresh material was used for anatomical studies whereas shade-dried material was powdered in an electric grinder for further studies. Four different types of Laata samples (nutritious herbal traditionalcompound formulations) were prepared separately by using 9 ingredients. Formulations composition of four herbalcompound formulationsof Laata aregiven in (Table1& Fig.2,3).

Materials and methods

Method forpreparation of laata sample-1(L1)

For preparation of the Latta sample-1four ingredients (Madhuca, Til, Alsi&Chirounji)were taken separately as per the defined quantity given in (Table 1). Clean and roast Madhuca flowers, Til seeds, Alsi seeds, and Chirounji seeds in a clay pot pan till become free from moisture, and prepare granulated powder separately. Weighed each ingredientseparately and mixedit in specific quantities to obtain a homogenous blendand prepared the Laata in the form of Laddu andgranular powder. Stored it in air-tight containers for further analysis.

Method forpreparation of laata sample-2(L2)

Latta Sample-2 was prepared by three ingredients *viz.*Madhuca flowers, Til seeds, and Gond (plant exude). Samples were taken separately as per the defined quantity given in (Table1). Clean and roast Madhuca flowers, Til seeds, and Gond (plant exude) in a clay pot pan till become free from moisture and prepare granulated powder separately. Weighed each ingredient separately and mixedit in a specific quantity to obtain a homogenous blend and prepared the Laata sample-2 in the form of Laddu and granular powder. Store it in air-tight containers for further analysis.

Method forpreparation of laata sample-3(L3)

Laata sample-3 was prepared with six ingredients. Samples were taken separately as per the defined quantity given in (Table 1). Cleaned and roasted Madhuca flowers in a clay pot pan till become free from moisture and prepared granulated powder separately. Cleaned, washed, and dried Til seeds, Alsi seeds, Chirounji seeds, Ashwagandha roots&Safed Musali rootstill became free from moisture and prepared granulated powder separately. Weighed each ingredient separately and mixedit in specific quantities to obtain a homogenous blend and prepared the Laata sample-3 in the form of Laddu and granular powder. Store it in air-tight containers for further analysis.

Method forpreparation of laata sample-4(L4)

Similarly,Laata sample-4 was prepared with eight ingredients. Samples were taken separately as per the defined quantity given in (Table 1). Cleaned and roasted Madhuca flowers in a clay pot pan till become free from moisture and prepared granulated powder separately. Cleaned, washed, and dried Til seeds, Alsi seeds, Chirounji seeds,

Ashwagandha roots,Safed Musali roots, Kaju fruits & Badam fruits till become free from moisture and prepared granulated powder separately. Weighed each ingredient separately and mixedthem in specific quantities to obtain a homogenous blend and prepared the Laata sample-4 in the form of Laddu and granular powder. Stored it in air-tight containers for further analysis.

Macroscopic study

Macroscopic or organoleptic characteristics of four herbal compound formulations of Laatalike colour, odour, and taste were evaluated.

Preparation of slides for powder microscopic study

Four different samplesof Latta were powderedseparately and completely passed through 355 µm IS Sieve (old sieve number 44) and not less than 50% passed on through 180 µm IS Sieve (old sieve number 85). About 2 g of powdersampleswere washedseparately thoroughly with potable water and poured out of the water without loss of material. Mounted a small portion of glycerin was used to all characteristics of thesamplesseparately, a small quantity of sampleswas cleared by heating with chloral hydrate solution, washed and mounted in glycerin, treated a few mg with iodine solution, and mounted in glycerin, another small quantity of samples stained with Sudan red solution and mounted with glycerin, all mounted slides were seen under microscope at 40 x 10x magnification of the Trinocular Research Microscopeseparately[8,9,10] .

Physico-chemical tests

Physico-chemical parameters such as moisture content (loss on drying at 105^0C), water-soluble extractive value, alcohol soluble extractive value, total ash value, acid insoluble ash value, and*pH* (10 %) aqueous solution were Performedin four samples of Laataseparately[11,12].

Nutritional Value Analysis

Nutritional value analysis has been performedonfour samples of Laata separately including parameters total calories, total carbohydrates, total fat,protein, vitamin C, dietary fiber, Iron (as Fe), Calcium (as Ca), vitamin A, Sodium (as Na) and Potassium (as K)[13,14].

Preliminary phyto-chemical analysis

Preliminary phyto-chemical tests of four samples of Laata separately were carried out on ethanolic and water extract for the presence of phytoconstituents like alkaloids, carbohydrates, flavonoids, protein, resins, saponin, tannin and steroids[15,16].

Screening of heavy metals tests

Heavy metals are toxic and generally occur throughoutthe earth in plants. The four main types of heavy metals harmful to us are Pb, Cd, As, and Hg. These heavy metals were detected through an Atomic Absorption Spectrophotometer as per standard method[17,18].

Pesticides residues analysis

There are 42 types of pesticides residues were analyzed in four samples of Laata separately, these are Alachlor mg/kg, Aldrin mg/kg, Dieldrin mg/kg, Azinphos methyl mg/kg, Bromopropylate mg/kg, Chlordane mg/kg, Chlorfenvinphosmg/kg, Chlorpyrifos mg/kg, Chlorpyrifos methyl, Cypermethrin mg/kg, 2,4-DDT mg/kg, 4,4-DDT mg/kg, 2,4-DDD mg/kg, 4,4-DDD mg/kg, 2,4-DDE mg/kg, 4,4-DDE mg/kg, Deltamethrin mg/kg, Diazinon mg/kg, Dichlorvos mg/kg, Dithlocarbamates mg/kg, Alpha-Endosulfan mg/kg, Endrin mg/kg, Ethion mg/kg, Fenitrothion mg/kg, Fenvalerate mg/kg, Fonofos mg/kg, Heptachlor mg/kg, Hexachlorobenzene mg/kg, Hexachlorocyclohexane Isomers mg/kg, Lindane mg/kg, Malathion mg/kg, Methidathion mg/kg, Parathion mg/kg,

Parathion methyl mg/kg, Permethrin mg/kg, Phosalone mg/kg, Piperonyl butoxide mg/kg, Pirimiphos methyl mg/kg, Pyrethrins mg/kg, Quintozene mg/kg, Beta-Endosulfan mg/kg, Endosulfan Sulphate mg/kg as per standard methods[19,20].

High-performance thin layer chromatography (HPTLC) fingerprint profile

For High-performance thin layer chromatography, 5 gm of each powdered sample (Four samples ID, L_1, L_2, L_3&L_4) were extracted with 100 ml of methanol overnight, filtered, and concentrated. A ferulic acid standard markerwasused forthe identification of Ferulic acid active phytochemical in Laata samples. For the preparation of standard marker working solutions, 10mg of Ferulic acid was dissolved in a 10 ml volumetric flask and made up the volume with methanol. Then transferred 1 ml from the stock solution to a 10 ml volumetric flask and made up the volume with methanol. From the solution, prepared standard solutions by transferring aliquots (0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 ml) corresponding to (1,2,3,4,5, and 6 ug/ml) of stock solution to 10ml volumetric flasks and made up the volume in each case to 10 ml with methanol.It was applied by spotting extracted samples on a pre-coated silica-gel aluminium plate 60 F_{254} (10x20 cm with 0.2 mm layer thickness Merk Germany) using a CamagLinomat -5 sample applicator and a 100 µlHamilton syringe. The samples and standard marker, in the form of bands of length 6 mm, were spotted 15 mm from the bottom, 15 mm from the left margin of the plate, and 10 mm part. Plates were developed using a mobile phase consisting of *toluene: ethyl acetate*: and *formic acid* (7:2.5: 0.5 v/v). Linear ascending development was carried out in a 20x20cm twin through a glass chamber equilibrated with a mobile phase. The optimized chamber saturation time for the mobile phase was 30 min. at room temperature. The length of the chromatogram run was 8 cm. 20 ml of the mobile phase. After the development, a thin layer of chromatography plate was dried with the help of a Hot Air Oven instrument. The peak area for samples and standard were recorded with the camera photo documentation system CamagReprostar3. Visualization of spotswas made before and after derivatization (with 5% Methanolic - sulphuric acid reagent) at 254nmand 366nm after derivatization with Win cat software and R_f values were noted[21,22].

Microbiological limit tests

Microbial limit tests for the estimation of the number of viable aerobic micro-organisms present and for detecting the presence of designated microbial species in pharmaceutical substances. The following tests were carried out as per[23] to determine the microbial load in four samples ofLaata.

Enumeration of *Staphylococcus aureus*/gm
Enumeration of *Salmonella sp*./gm
Enumeration of *Pseudomonasaeruginosa*/gm
Enumeration of *Escherichia coli*
Determination of total microbial count (TBC)
Determination of Yeast &Mould

The microbiological tests were determined using specified agar media and enrichment media from Himedia, Pvt. Ltd. Mumbai.

Results

Macroscopic characters

A blackish brown LaataLadduand powder with the characteristic odour of Madhuca and a light sweetish taste(Fig. 4).

Powder microscopic characters

***Laata sample-1(L1)*:**Pollen grains, fibrous layer of the anther, epidermal cells of petals with striated cuticle, covering trichomes, fragments of pigment vessels, glandular trichomes (Mahua); fragment of endosperm tissue in surface view embedded with aleurone grains and oil globules, parenchyma tissue with aleurone grains, the fragment of the cotyledon (Til);Epidermis of testa in surface view

overlapping with underlined parenchymatous cells, a fragment of cotyledon embedded with fixed oil globules and aleuron grains, fragment of pigment cells layer in surface view, a fragment of sclerenchymatous cells in surface view (Alsi); Various types of sclereids, pigment cells of testa in surface view, pigment layer of testa overlapping with endosperm in surface view, upper epidermis of cotyledon overlapping with collapsed celled layer and vessels in surface view (Chiroungi) (Fig. 5).

Laata sample-2(L2): Pollen grains, fibrous layer of anther, epidermal cells of petals with striated cuticle, covering trichomes, fragments of pigment vessels, glandular trichomes (Mahua); fragment of endosperm tissue in surface view embedded with aleurone grains and oil globules, parenchyma tissue with aleurone grains, fragment of cotyledon (Til) (Fig. 5).

Laata sample-3(L3): Pollen grains, fibrous layer of anther, epidermal cells of petals with striated cuticle, covering trichomes, fragments of pigment vessels, glandular trichomes (Mahua); fragment of endosperm tissue in surface view embedded with aleurone grains and oil globules, parenchyma tissue with aleurone grains, fragment of cotyledon (Til);Epidermis of testa in surface view overlapping with underlined parenchymatous cells, fragment of cotyledon embedded with fixed oil globules and aleuron grains, fragment of pigment cells layer in surface view, fragment of sclerenchymatous cells in surface view (Alsi); Various types of sclereids, pigment cells of testa in surface view, pigment layer of testa overlapping with endosperm in surface view, upper epidermis of cotyledon overlapping with collapsed celled layer and vessels in surface view (Chiroungi);cortical parenchymatous cells and medullary rays cells containing simple starch grains that are up to 30µ, mular shaped, mostly single, occasionally groups of two or three with slit like or stellate hilum(Ashwagandha);Fragments of beaded or pitted thick walled cells of epiblema in surface view with prismatic crystals of calcium oxalate, parenchymatous cells of ground tissue filled with raphides, a few root hairs (Musali) (Fig.5).

***Laata sample- 4(L4)*:** Pollen grains, fibrous layer of anther, epidermal cells of petals with striated cuticle, covering trichomes, fragments of pigment vessels, glandular trichomes (Mahua); fragment of endosperm tissue in surface view embedded with aleurone grains and oil globules, parenchyma tissue with aleurone grains, fragment of cotyledon (Til);Epidermis of testa in surface view overlapping with underlined parenchymatous cells, fragment of cotyledon embedded with fixed oil globules and aleuron grains, fragment of pigment cells layer in surface view, fragment of sclerenchymatous cells in surface view (Alsi); Various types of sclereids, pigment cells of testa in surface view, pigment layer of testa overlapping with endosperm in surface view, upper epidermis of cotyledon overlapping with collapsed celled layer and vessels in surface view (Chiroungi);cortical parenchymatous cells and medullary rays cells containing simple starch grains that are up to 30µ, mular shaped, mostly single, occasionally groups of two or three with slit like or stellate hilum(Ashwagandha);Fragments of beaded or pitted thick walled cells of epiblema in surface view with prismatic crystals of calcium oxalate, parenchymatous cells of ground tissue filled with raphides, a few root hairs (Musali); Mesophyll cells in surfaces view filled with starch grains, aleurone grains & oil globules, Oil globules embedded within mesophyll cells (Kaju);Stone cells, Reticulate parenchyma, Endosperm cells filled with aleurone grains & oil globules (Badam) (Fig. 5)

Physico-chemical analysis

The physicochemical parameters such as extractive values are useful for the determination of exhausted or adulterated drug;

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ash values of the drug gave an idea of the earthy matter or the inorganic composition and other impurities present along with the drug, Loss on drying at 105^0C and pH (10 %) aqueous solution was performed. Four samples of Laataphysico-chemical resultsare given in (Table 2)

Nutritional value analysis

Nutritional value analysis *viz.*total calories, total carbohydrates, total fat,protein, Vitamin C, dietary fiber, Iron (as Fe), Calcium (as Ca), Vitamin A, Sodium (as Na), and Potassium (as K)offour samples of Laata were done separately.Nutritional value analysis results are given in (Table 3).

Preliminary phyto-chemical investigation

Qualitative phyto-chemical analysis wasperformed in water and ethanol extracts. Alkaloids, carbohydrates, flavonoids, protein, resin, and saponin were present in Laata samples 1,2, and 3 whereas carbohydrates, flavonoids, protein, resin, saponin, tannin, alkaloids, and steroids were present in sample 4.

Heavy metals tests

Heavy metal elements (Pb, Cd, As, and Hg) tests were performed and found under limits as per guideline WHO, and results are given in (Table 4).

Microbiological limit tests

Microbiological analysis of pathogenic bacteria, *viz.Salmonella* sp., *Escheria coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were done and found that absent in Laata samples were total microbial plate count (TPC) was found 35cfu/g in sample 1, 94cfu/g in sample 2, 15cfu/g in sample 3, 60 cfu/g in sample 4 and yeast &moulds found 85 cfu/g in sample1, 56cfu/g in sample 2, 10 cfu/g in sample 3 and 80cfu/g in sample 4. The microbiological profile of the Laata four samples was found satisfactory under prescribed limits such as for *Salmonella* sp., *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* limits were absent, whereas fortotal microbial plate count (TPC)10^5cfu/g and for yeast &moulds 10^3cfu/gas per WHO guidelines.

Pesticides residues analysis

There are 42 types of pesticide residues *viz.*Alachlor mg/kg, Aldrin mg/kg, Dieldrin mg/kg, Azinphos methyl mg/kg, Bromopropylate mg/kg, Chlordane mg/kg, Chlorfenvinphosmg/kg, Chlorpyrifos mg/kg, Chlorpyrifos methyl, Cypermethrin mg/kg, 2,4-DDT mg/kg, 4,4-DDT mg/kg, 2,4-DDD mg/kg, 4,4-DDD mg/kg, 2,4-DDE mg/kg, 4,4-DDE mg/kg, Deltamethrin mg/kg, Diazinon mg/kg, Dichlorvos mg/kg, Dithlocarbamates mg/kg, Alpha-Endosulfan mg/kg, Endrin mg/kg, Ethion mg/kg, Fenitrothion mg/kg, Fenvalerate mg/kg, Fonofos mg/kg, Heptachlor mg/kg, Hexachlorobenzene mg/kg, Hexachlorocyclohexane Isomers mg/kg, Lindane mg/kg, Malathion mg/kg, Methidathion mg/kg, Parathion mg/kg, Parathion methyl mg/kg, Permethrin mg/kg, Phosalone mg/kg, Piperonyl butoxide mg/kg, Pirimiphos methyl mg/kg, Pyrethrins mg/kg, Quintozene mg/kg, Beta-Endosulfan mg/kg, Endosulfan Sulphate mg/kgwere analyzed in four samples of Laata separately, these are Alachlor mg/kg, Aldrin mg/kg, Dieldrin mg/kg, Azinphos methyl mg/kg, Bromopropylate mg/kg, Chlordane mg/kg, Chlorfenvinphosmg/kg, Chlorpyrifos mg/kg, Chlorpyrifos methyl, Cypermethrin mg/kg, 2,4-DDT mg/kg, 4,4-DDT mg/kg, 2,4-DDD mg/kg, 4,4-DDD mg/kg, 2,4-DDE mg/kg, 4,4-DDE mg/kg, Deltamethrin mg/kg, Diazinon mg/kg, Dichlorvos mg/kg, Dithlocarbamates mg/kg, Alpha-Endosulfan mg/kg, Endrin mg/kg, Ethion mg/kg, Fenitrothion mg/kg, Fenvalerate mg/kg, Fonofos mg/kg, Heptachlor mg/kg, Hexachlorobenzene mg/kg, Hexachlorocyclohexane Isomers mg/kg, Lindane mg/kg, Malathion mg/kg, Methidathion mg/kg, Parathion mg/kg,

Parathion methyl mg/kg, Permethrin mg/kg, Phosalone mg/kg, Piperonyl butoxide mg/kg, Pirimiphos methyl mg/kg, Pyrethrins mg/kg, Quintozene mg/kg, Beta-Endosulfan mg/kg, Endosulfan Sulphate mg/kgwere analyzed in four samples of Laata separately and found below limit of quantification.

HPTLC (high-performance thin-layer chromatography) fingerprints profile

High-performance thin layer chromatography (HPTLC) study of the methanolic extractsin four spots of the Laata four samples extract and Ferulic acid standard marker in six spots applied in precoated TLC plate. Applied 6µl of the test solution as well as standard marker as 6 mm bands and developed the plate in a solvent system *toluene: ethyl acetate*: *formic acid* (7:2.5: 0.5 v/v) to a distance of 8 cm. Dried the developed plate in room temperature and examined. Derivatized the plate using 5% *Methanolic-sulphuric acid*reagent and dried at 105^0C ina Hot air oven, till the bands were visible. Major spots $R_f$values with colour were recorded before derivatization at254nmand after derivatization at 366nm. Major spots of R_f values at 254nm before derivatization are 0.08 light black, 0.20black, 0.24 black, 0.40 light black, 0.56black with Ferulic acid standard, and after derivatization at 366nm R_f values are 0.06 sky blue, 0.56 sky blue with Ferulic acid standard, 0.60 brown, 0.70, sky blue and 0.80 sky blue.A Chromatogram profile is given(Fig.6).

Discussion

For the present study, a systematic survey and documentation of traditional knowledge with special reference to Laata (a nutritious herbal compound formulation) used by villagers in the Chitrakoot region of 24 tribal (Kol, Gond, Mawasi &Khairwar) dominant villages surveyed, a total 760 interviews conducted in 98 intensive field visits were carried out during 2019-2020, covering almost all the seasons of the year. The 45-80 years traditional healers, local vaidyas, especially experienced women who have been actively engaged in the preparation of Laata (a traditional nutritious recipe)as well as ethnomedicinal practicesThe health practices whether they are related to traditional food formulations described in the study involved total 9 plant species belonging to 6 different families are used forthe preparation of four types of Laata (a nutritious herbal compound formulation). Out of 9 plant species4 are herbs and 4 trees, various parts of plants such as flowers 1plant, fruits 2 plants, seeds 3 plants, roots 2 plants and exude1 plantwere used as mentioned in Table1in preparations of 4 different formulations of Laata[24,25,26].

As per the pharmacognostic study of the Laata Namely Laatasample1 (L1), Laatasample2 (L2), Laatasample3 (L3), and Laatasample4 (L4) were tested for relevant microscopical, physical, and chemical parameters. The formulations were subjected to various analytical techniques. Organoleptic parameters revealed that the tested 4samplesare blackish brown with the characteristic odour of Madhuca and a light sweetish taste.Powder microscopic tests were performed and established the distinguished anatomical characters for four different Laata samples, results are depicted (Fig.5), and these specific anatomical characters may be helpful for the identification of ingredients in the Laata[27].

Qualitative tests for four samples of Laata were performed *viz.*loss on drying at 105^OC, total ash content, acid insoluble ash, water soluble extractive, alcohol soluble extractive, and *pH* (10 %w/v) aqueous solutionwere found to be within standards range.

The results of the physicochemical analysis are given in (Table 2). The results are expressed as mean (n=3)+- standard deviation. The total ash value is indicative of a total amount of inorganic material after complete incineration and the acid insoluble ash value is indicative of

silicate impurities, which might have arisen due to improper washing of the ingredients. Ash value is useful in determining the authenticity and purity of the drug and also these values are important quantitative standards,the extractive values, alcohol-soluble, and water-soluble indicate the number of active constituents in a given amount of plant material when extracted with a respective solvent. The loss on drying value obtained is indicative of the amount of moisture content that could prevent bacteria, fungal, or yeast growth,The *pH* of the 10% w/v aqueous solution revealed that the four Laata samples are acidic.

Qualitative phyto-chemical analysis was performed in water and ethanol extracts. Alkaloids, carbohydrates, flavonoids, protein, resin, and saponin were present in Laata samples 1(L1), 2(L2), and 3(L3), where carbohydrates, flavonoids, protein, resin and saponin, tannin, alkaloids, and steroids were present in sample 4(L4)which could make the formulation useful for potential and preventive healthcare needs[28].Pesticide residues were analyzed in four samples of Laata separately and found below the limit of quantification.HPTLC chromatograms of the four samples of Laata nutritious herbal formulation with Ferulic acid standard. The $R_f$values andcolour of the resolved bands are noted. Developed chromatograms indicate the presence of Ferulic acid standards in Laata samples. This confirms the batch-to-batch consistency of the finished products and can serve as the quality standard for manufacturers of the same formulation in the future. Microbiological analysis of pathogenic bacteria, *viz.Salmonella* sp., *Escheria coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were done and found that absent in Laata samples were total microbial plate count (TPC) was found 35cfu/g in sample 1(L1), 94cfu/g in sample 2(L2), 15cfu/g in sample 3(L3), 60 cfu/g in sample 4(L4) and yeast &moulds found 85 cfu/g in sample1, 56cfu/g in sample 2, 10 cfu/g in sample 3 and 80cfu/g in sample 4. The microbiological profile of the Laata four samples was found satisfactory under prescribed limits such as for *Salmonella* sp., *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* limits were absent, whereas fortotal microbial plate count (TPC)10^5cfu/g and for yeast &moulds 10^3cfu/gas per WHO guidelines, these results indicate that samples are free from harmful bacteria.Similarly,the detection of heavy metals (Pb, Cd, As & Hg) and 42 types of pesticide residue tests were performed and found under limits/absent as per WHO guidelines[29].

Nutritional value tests of four compound formulations of Laata were analyzed such as the total calories range of four samples of Laata 395.10-469.91kcal/100gm, total carbohydrates range 45.60-57.18g/100gm, total fat 17.40-22.63g/100gm, protein 10.75-13.56g/gm, vitamin c 2.00-3.39mg/100gm, dietary fibre 3.90-4.89g/100gm, Iron 8.60-10.48mg/gm, Calcium 12.50-17.72mg/gm, Sodium 62.34-80.20mg/gm and Potassium 105.60-155.70mg/gm were found[30]. These findings are indicationsthatLaata (a traditional herbal nutritious compound formulation) is a very good traditional herbal nutritious recipe used by the tribal of the Chitrakoot region to maintain good health. These practices if integrated with the modern healthcare system could elevate the health status of thousands of rural people as well as urban people. This study may also be helpful in the preparation of Laata and their quality evaluation.

Conclusion

From the present investigation,documentation of folklore practices and pharmacognostic study with special reference to Laata at Chitrakoot region of MadhyaPradeshwere performed. It includes various parameters such as documents of traditional knowledge, selection of formulations, and its importance as

nutritious food materials used by tribal of the Chitrakoot region as a breakfast recipe for energy, nutritional, and healthy life. Macroscopy, powder microscopic study, nutritional value analysis, physicochemical analysis, phytochemical investigation, heavy metal testing, microbiological assays, pesticides residue analysis, and development of HPTLC fingerprints profile with quantification of Ferulic acid marker werecarried out as per Ayurvedic Pharmacopoeial standards. The results of the present study showed satisfactory as the efficacy and potency of the products as immunoboosters, nutritious and to attain life-long health goals. The developed products can afford the health care needs in society. The research outcomings of the documentation and standardization parameters can be used for evaluation of the quality and purity of the Laata (a traditional nutritious herbal compound formulation) and also serve as a reference monograph in the preparation of food supplementary products.

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Conflict of interest

The authors declare no conflict of interest.

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Table 1- Formulation Composition of Laata

S. No	Formulations name Laata sample 1 (L1)	Botanical name	Part used	Quantity
1.	Mahua	*Madhuca longifolia* (J. Koenig ex L.) J.F. Macbr(Sapotaceae)	Flower	6part
2.	Til	*Sesamum indicum* L. (Pedaliaceae)	Seed	3.0part
3.	Alasi	*Linum usitatissimum*L. (Linaceae)	Seed	0.5part
4.	Chirounji	*Buchananiacochinchinensis* (Lour.) M.R. Almeida (Anacardiaceae)	Seed	0.5part
	Laata sample 2 (L2)			
5.	Mahua	*Madhuca longifolia* (J. Koenig ex L.) J.F. Macbr(Sapotaceae)	Flower	6part
6.	Til	*Sesamum indicum* L. (Pedaliaceae)	Seed	2part
7.	Gond	*Vachelliainlotica*subsp*indica* (Benth.) Kyal. &Boatwr. (Fabaceae)	Exude(plants)	2part
	Laata sample 3 (L3)			
8.	Mahua	*Madhuca longifolia* (J. Koenig ex L.) J.F. Macbr(Sapotaceae)	Flower	5part
9.	Til	*Sesamum indicum* L. (Pedaliaceae)	Seed	3part
10.	Alasi	*Linum usitatissimum*L. (Linaceae)	Seed	0.5part
11.	Chirounji	*Buchananiacochinchinensis* (Lour.) M.R. Almeida (Anacardiaceae)	Seed	0.5part
12.	Ashwagandha	*Withaniasomnifera*(L.) Dunal (Solanaceae)	Root	0.3part
13.	Safed Musali	*Chlorophytum arundinaceum*Baker(Asparagaceae)	Root	0.7part
	Laata sample 4 (L4)			
14.	Mahua	*Madhuca longifolia* (J. Koenig ex L.) J.F. Macbr(Sapotaceae)	Flower	5part
15.	Til	*Sesamum indicum* L. (Pedaliaceae)	Seed	2part
16.	Alasi	*Linum usitatissimum*L. (Linaceae)	Seed	0.5part
17.	Chirounji	*Buchananiacochinchinensis* (Lour.) M.R. Almeida (Anacardiaceae)	Seed	0.5part
18.	Ashwagandha	*Withaniasomnifera*(L.) Dunal (Solanaceae)	Root	0.3part
19.	Safed Musali	*Chlorophytum borivlianum (Roxb.)* (Liliaceae)	Root	0.7part
20.	Kaju	*Anacardium occidentale* L. (Anacardiaceae)	Fruit	0.5part
21.	Badam	*Prunus amygdalus* Batsch. (Rosaceae)	Fruit	0.5part

Table 2- Physico-chemical parameters of Laata

S. No	Parameters	Sample 1(L1)	Sample 2(L2)	Sample 3(L3)	Sample 4(L4)
1	Loss on drying at105° C	6.59	5.0	6.1	5.59
2	Total Ash	9.50	8.64	11.34	12.50
3	Acid – insoluble ash	1.34	1.23	1.2	1.33
4	Alcohol-soluble extractive	51.63	49.75	50.26	50.58
5	Water-soluble extractive	49.16	51.45	52.03	51.28
6	*pH (10 %) aqueous solution*	5.0 to 6.0	5.0 to 6.0	5.0 to 6.0	5.0 to 6.0

Table3-Nutritional Value Analysis of Laata

S. No	Parameters	Unit of measurement	Sample 1(L1)	Sample 2(L2)	Sample 3(L3)	Sample 4(L4)
1	Total Calories	Kcal/100g	469.91	395.10	465.30	459.66
2	Total carbohydrates	g/100g	53.00	45.60	54.70	57.18
3	Total fat	g/100g	22.63	17.40	21.50	19.94
4	Protein	g/100g	13.56	10.75	13.35	12.87
5	Vitamin C	mg/100g	3.39	2.00	3.10	2.13
6	Dietary fiber	g/100g	4.53	3.90	4.35	4.89
7	Iron (as Fe)	mg/100g	10.48	8.60	10.0	10.43
8	Calcium (as Ca)	mg/100g	17.72	12.50	14.20	14.00
9	Vitamin A	IU/100gm	10.0	10.0	10.0	10.0
10	Sodium (as Na)	mg/100g	80.20	62.34	75.20	72.50
11	Potassium (as K)	mg/100g	155.70	105.60	135.40	140.80

Table4- Determination of Heavy Metals in Laata

S. N.	Parameters	Sample 1 (L1)	Sample 2 (L2)	Sample 3 (L3)	Sample 4 (L4)	API Limits
1	Lead (Pb)	0.4103ppm	0.4173ppm	0.4123 ppm	0.5430 ppm	10 ppm
2	Cadmium (Cd)	0.0529ppm	0.0596ppm	0.0321 ppm	0.04539 ppm	0.3 ppm
3	Arsenic (As)	0.4346ppb	0.6128ppb	0.5697ppb	0.5631ppb	03 ppm
4	Mercury (Hg)	0.5156ppb	0.5432ppb	0.5236ppb	0.5672ppb	01 ppm

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Fig.1-Documentation of traditional knowledge

Fig.2-Process of Laata preparation

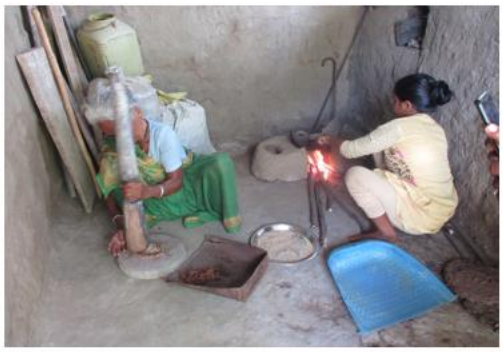
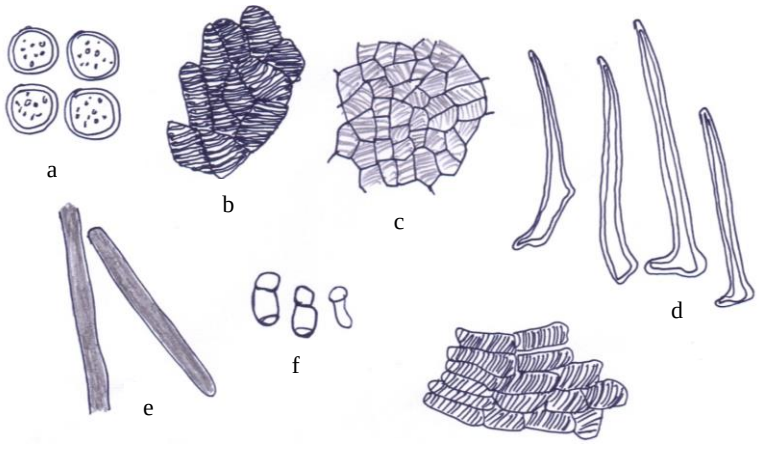


Fig.3-Preparation of Laata

Fig.4-Finished products of Laata



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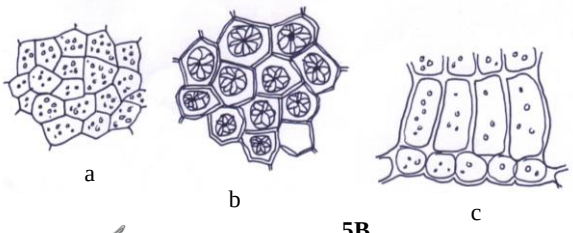

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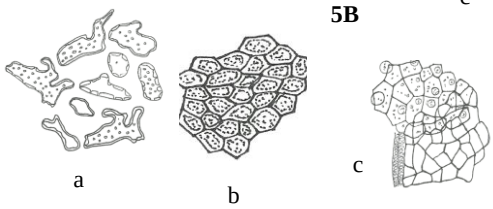

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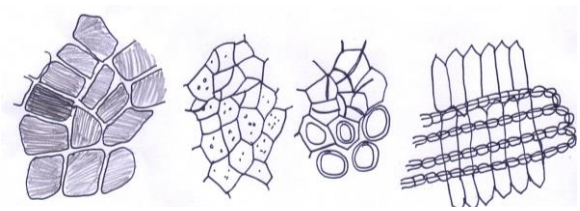

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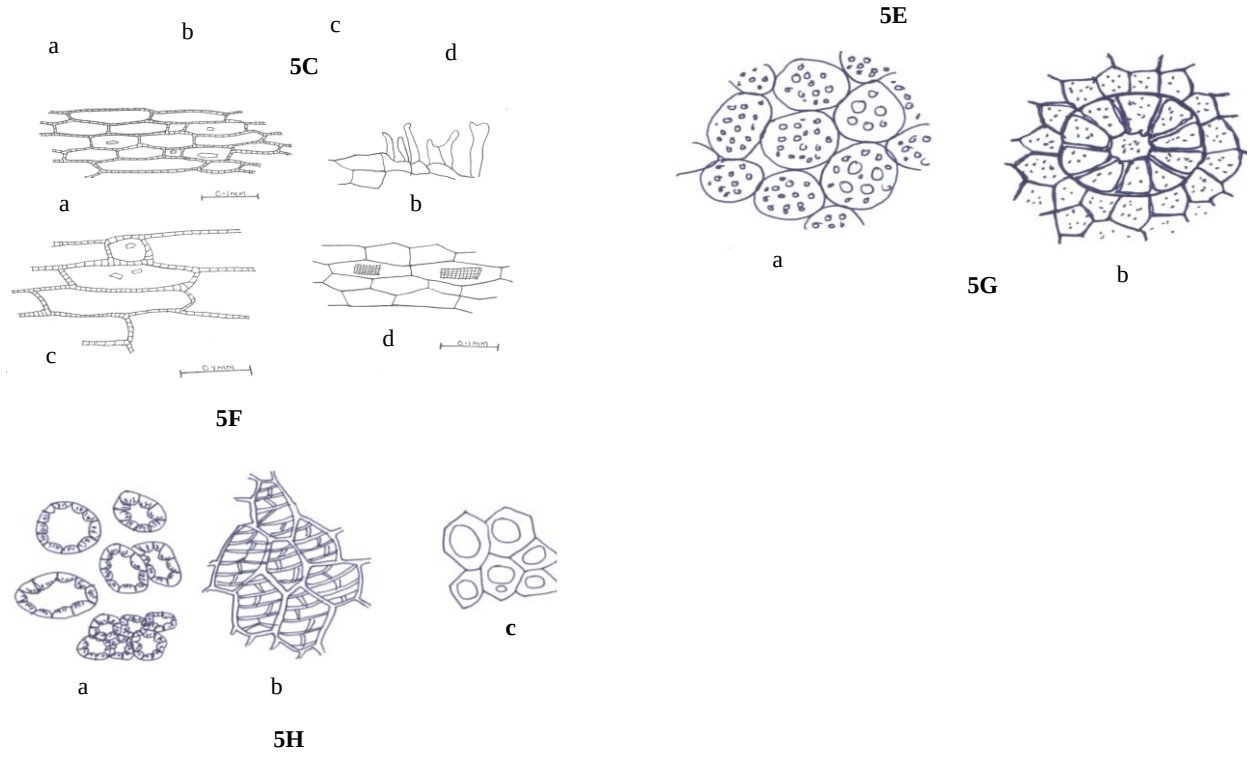

Fig.5- Powder microscopy of Laata four samples (5A) Madhuca (a) Pollen grains (b) Fibrous layer of anther (c) Epidermal cells of petals with striated cuticle (d) Covering trichomes (e) Fragments of pigment vessels (f) Glandular trichomes (g) Fibrous layer of anther (5B) Til (a) Endosperm tissue (b) Parenchyma tissue with aleurone grains (c) Fragment of cotyledon (5C)Alsi (a) Pigment cells layer in surface view (b) Fragment of cotyledon embedded with fixed oil (c) Epidermis of testa (d) Fragment of sclerenchymatous cells in surface view (5D) Chirounji (a) sclereids (b) Pigment cells of testa in surface view (c) Upper epidermis of cotyledon overlapping with collapsed celled layer and vessels in surface view (5E)Ashwagandha (a) Parenchyma containing sandy crystals and starch grains (b) Medullary rays (5F) Musal (a) Epiblema in surface view with prisms (b) root hairs (c) Epiblema in surface view with prisms (d) Parenchyma filled with raphids (5G) Kaju (a) Mesophyll cells in surfaces view filled with starch grains, aleurone grains & oil globules (b) Oil globules embedded within mesophyll cells (5H) Badam (a) Stone cells (b) Reticulate parenchyma (c) Endosperm cells filled with aleurone grains & oil globules

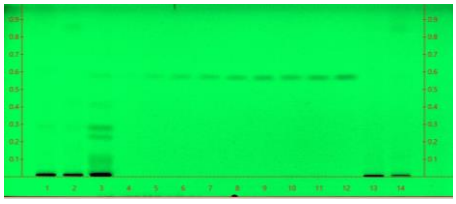

Fig. 6A-HPTLC fingerprints profile of Laata samples with Ferulic acid standard marker at 254nm before derivatization

Abbreviations- track 1&2 Laata sample 1(L1), track 3 Laata sample 3(L3),track 4 to 12 Ferulic acid standards marker, track 13 Laata sample 3(L3) &track 14 Laatasample 4(L4).

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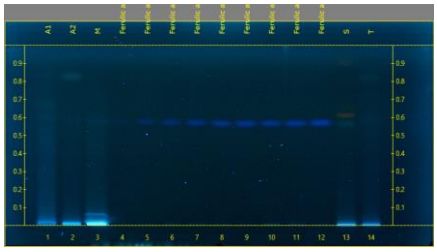

Fig. 6B-HPTLC fingerprints profile of Laata samples with Ferulic acid standard marker at 366nm after derivatization

Abbreviations- track 1&2 Laata sample 1(L1), track 3 Laata sample 3(L3),track 4 to 12 Ferulic acid standards marker, track 13 Laata sample 3(L3) & track 14 Laata sample 4(L4).