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DOCUMENTATION AND STANDARDIZATION OF JANMAGHUTTI-A TRADITIONAL HERBAL FORMULATION FOR CHILD HEALTHCARE IN THE CHITRAKOOT REGION OF MADHYA PRADESH

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Chitrakoot is a remote region situated in the northern part of Satna district of Madhya Pradesh, India. The tribals (Kol, Gond, Mawasi, Khairwar) of this region have well-developed folklore practices used to maintain basic healthcare needs. A survey was carried out among the tribal community of the Chitrakoot region of Satna district, Madhya Pradesh to explore the traditional knowledge on the use of Janmghutti (a traditional medicinal formulation) using semi-structured interviews among the local healers and elderly women ranging in age group of 45-80 years. A total of 757 interviews conducted in 98 intensive field visits were carried out during 2019-2020, covering almost all the seasons of the years, who have been actively engaged in preparation of Janmaghutti. A total of 11 plant ingredients, belonging to 8 families and 3 other ingredients were found to be used to make Janmaghutti powder. As per the standardization parameters of AYUSH viz. macroscopy, powder microscopy, physicochemical tests, heavy metals test, pesticides residue tests, screening of microbiological species, and high-performance thin layer chromatography (HPTLC) fingerprints of methanolic extract were performed and nutritional value analysis was also done. Macroscopy and microscopy characters establish the internal characteristics of Janmaghutti formulation and its ingredients. Physicochemical parameters, heavy metals (Pb, Cd, As & Hg), and 42 types of pesticide residue tests were performed and found as per the limits/absence of WHO guidelines. Microbiological analysis of pathogenic bacteria, viz. Salmonella sp., Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus were done and found that absent in Janmaghutti samples. Nutritional value analysis of two compound formulations of Janmaghutti samples (JI & J2) were done such as total fat % by wt. 11.7%, 16.8%, Protein (N*5.95) % by wt. 6.7%, 9.4%, Crude fibre % by wt. 3.4%, 3.2%, Total dietary fibre % 16.44%, 14.13%, Carbodydrates by difference, % by wt. 44.2%, 43.0%, Energy value K cals/100mg 310, 361, Vitamin D mg/100g 0.01, 0.01, Sodium mg/100gm 3601.30,3239.30, Calcium mg/100gm 478.8, 413.80, Iron mg/100g 19.28, 16.70, Potassium mg/100gm 1070.87 and 1020.50 were found respectively. These findings indicate that Janmaghutti (a traditional formulation) is a very good traditional herbal formulation used by tribal communities of the Chitrakoot region to maintain resistant power and immunity against common ailments of children. In the future, it can be used as a strength promoter and immune modulator and will enhance the healthcare system not only the rural India but also in urban societies.

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Keywords: Folklore knowledge, HPTLC fingerprinting, Janmaghutti, Nutritional value, Standardization.

Introduction

The use of plants or animal products for healing is as old as human civilization. Medicinal plants continue to be an important therapeutic aid for alleviating ailments of mankind[1]. Approximately 80% of the people in developing countries rely on traditionally used medicinal plants for their primary health care needs. Plants are a rich source of bioactive compounds (secondary metabolites) and are of great value for developing novel therapeutic agents[2]**.** Due to accessibility to modern health care, the real knowledge of the uses of plants lies with the rural population of the country

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consisting of tribal, forest dwellers and many villagers.

India has a vast store of oral medical knowledge available in the form of tribal medicine, home remedies, and local health traditions. The people living in remote areas who are untouched by modern civilization use plants for their basic health care need[3-5].

Chitrakoot is 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. 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[6]**.** 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[7]. In this concern, a study has been undertaken to document folklore practices with special reference to Janmaghutti (a traditional herbal medicinal formulation), used in almost all households for centuries to improve the digestive power and strengthen the immune system of babies.

Therefore, it is important to develop better standardization techniques for the validation of traditional medicine and quality control standards of the plants used in indigenous systems of medicine. For standardization of the Janmaghutti, the study deals with the morphological, anatomical evaluation, physicochemical tests, preliminary phytochemical screening, heavy metals test, pesticide residue tests, microbiological screening, High-Performance Thin Layer Chromatography, and nutritional value analysis.

Materials and methods

Systematic survey and documentation of folk-lore/traditional knowledge

For the present study, a systematic survey and documentation of traditional knowledge with special reference to Janmaghutti as used by tribal communities (Kol, Gond, Mawasi & Khairwar) of Chitrakoot region was carried out in 24 tribal villages 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 757 men and women were interviewed in 98 intensive field visits during 2019-2020, covering almost

all the seasons. The 45-80-year-old traditional healers, local vaidyas, and especially experienced women who have been actively engaged in the preparation of Janmaghutti were interviewed and their prior informed consent was recorded. Detailed information regarding preparation and uses of the formulation such as plant (ingredients) name, parts used, the ratio of ingredients, mode of preparation, mode of application, doses, duration, benefits, and age groups, etc. have 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 in a research laboratory, Arogyadham, Deendayal Research Institute, Chitrakoot, dist. Satna (M.P.) (Fig.1,2).

Collection of samples and formulation preparation

The fresh ingredients of the Janmaghutti formulation were collected from the Chitrakoot forest viz. Haritaki -Terminalia chebula Retz. (Combretaceae) fruits, Bibhitaki- Terminalia bellirica (Gaertn.) Roxb. (Combretaceae) fruits, Haldi- Curcuma longa L. (Zingiberaceae) rhizome, Śunthî -Zingiber officinale Rosc. (Zingiberaceae) rhizome, Ajowan -Trachyspermum ammi (L.) Sprague (Apiaceae) fruit, Jatīphala- Myristica fragrans Houtt. (Myristicaceae) fruit, Hingu- Ferula assa-foetida L. (Apiaceae) Exude, Sauvarcala (black salt), Saindhava (rock salt), Kaju - Anacardium occidentale L. (Anacardiaceae) fruits, Badam (Prunus amygdalus Batsch (Rosaceae) fruits, Chhuhara -Phoenex dactylifera L. (Arecaceae) fruit, Draksha- Vitis vinifera L. (Vitaceae) fruits. Samples were identified and authenticated by a Senior Scientist of Deendayal Research Institute, Chitrakoot, Fresh material was used for anatomical studies whereas shade-dried material was powdered in an electric grinder for further studies. Two different types of Janmaghutti were prepared separately by using 11 ingredients. Formulations composition of two herbal compound formulations of Janmaghutti is given in (Table 1 & Fig 1).

Method for preparation of Janmaghutti sample-1(J1)

For preparation of the Janmaghutti sample-1 (J1) ingredients (Sunthi, Ajowan, Haritaki, Bibhitaki, Jatiphala, Haldi, Hingu, Sauvarcala, and Saindhava) were taken separately as per the defined quantity given in (Table 1). Clean, wash, dry, and powdered the samples separately. Weighed each ingredient separately and mixed it in a specific quantity and enough to pass through 355 µm IS Sieve (old sieve number 44) to obtain a homogenous blend. Store it in air-tight containers for further analysis.

Method for preparation of Janmaghutti sample-2(J2)

Similarly, Janmaghutti sample-2 (J2) is prepared by 13 ingredients (viz. Sunthi, Ajowan, Haritaki, Bibhitaki, Jatiphala, Haldi, Hingu, Sauvarcala, Saindhava, Kaju, Badam, Chohara and Draksha) were taken separately as per defined quantity given in (Table 1). Clean, wash, dry, and powdered the samples separately. Weighed each ingredient separately and mixed it in a specific quantity and enough to pass through 355 µm IS Sieve (old sieve number 44) to obtain a homogenous blend. Store it in air-tight containers for further analysis[8].

Macroscopic study

Organoleptic characters or macroscopic of Janmaghutti samples like colour, odour, and taste were evaluated.

Preparation of slides for powder microscopic study of Janmaghutti

About 50 gm of powder in each sample (J1& J2) was taken on a 106 µm IS Sieve used lukewarm water from a wash bottle sprayed a jet onto the material, stirred the samples from side to side simultaneously used a scalpel so that the jet of water removed dissolve salts through the sieve. Repeat the process 3 times. Collect the materials left on the sieve separately. Mounted a small portion in glycerin; warmed a few mg with chloral hydrate solution, washed in water, and mounted in glycerin; treated a few mg with iodine in potassium iodide solution and mounted in glycerin; heated a few mg in 2 percent aqueous potassium hydroxide, washed in water and mounted in glycerin. All mounted slides were seen separately under the microscope at 40 x 10x magnification of the Trinocular Research Microscope separately[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 Performed in two samples of Janmaghutti (J1 & J2) separately[11, 12].

Nutritional value analysis

Nutritional value analysis has been performed on two samples of Janmaghutti (J1 & J2) separately in various parameters such as total fat % by weight, Protein (N*5.95) % by weight, Crude fibre % by weight, Total dietary fibre % Carbohydrates by difference, % by weight, Energy value K cals/100mg, Vitamin D -mg/100g, Sodium -mg/100gm, Calcium- mg/100gm, Iron -mg/100g and Potassium- mg/100gm as per standard methods[13,14].

Preliminary phytochemical analysis

Preliminary phytochemical analysis of two samples of Janmaghutti (J1 & J2) were performed on ethanolic extract and water extract for the presence of phyto-constituents like alkaloids, carbohydrates, flavonoids, protein, resins, saponin, tannin and steroids separately [15, 16].

Screening of heavy metals tests

Heavy metals are toxic and generally occur throughout the 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

42 types of pesticides residues were analyzed in two samples of Janmaghutti (J1 & J2) separately, these are Alachlor mg/kg, Aldrin mg/kg, Dieldrin mg/kg, Azinphos methyl mg/kg, Bromopropylate mg/kg, Chlordane mg/kg, Chlorfenvinphos mg/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 sample (J1 & J2) powder was extracted with 100 ml of methanol overnight, filtered, and concentrated. Ferulic acid, Kaempferol, and Caffeic acid standard markers were used for the identification of
Ferulic acid, Kaempferol, and Caffeic acid are active phytochemicals in Janmaghutti samples (J1 & J2). For the preparation of standard marker working solutions, 10mg of Ferulic acid, Kaempferol, and Caffeic acid were dissolved in a 10 ml volumetric flask and made up the volume with methanol separately. Then 1 ml of stock solution is transferred to a 10 ml volumetric flask and made up the volume with methanol separately. 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 separately. These were applied by spotting extracted samples (J1 & J2) on pre-coated silica-gel aluminium plate 60 F_{254} (10x20 cm with 0.2 mm layer thickness Merk Germany) using Camag Linomat -5 sample applicator and a 100 µl Hamilton syringe. The samples and standard markers, 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 (7:5v/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 standards was recorded with the camera photo documentation system Camag Reprostar 3. Visualization of spots was made before and after derivatization (with 5% Methanolic - sulphuric acid reagent) at 254nm and 366nm after deivatization 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. Following tests were carried out to determine the microbial load in two samples of Janmaghutti (J1 & J2)[23, 24].
Enumeration of Staphylococcus aureus/gm
Enumeration of Salmonella sp./gm
Enumeration of Pseudomonas aeruginosa/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

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A brownish-yellow powder, with the characteristic odour of Hingu and with a salty taste. The powder completely passes through 355 µm IS Sieve (old sieve number 44) and not less than 50 percent passes through 180 µm IS Sieve (old sieve number 85).

Powder microscopic characters

Janmaghutti sample-1(J1)

Parenchymatous cells containing oleo-resin, oval to round starch grains not less than 15µ to 30µ and several up to 70µ with hilum ecentric, lamellae distinct; pitted, septate fibres with indentations on its walls (Śunthî); epidermis with papillose and glandular out growths with cuticular striations radiating from base (Ajowan); Thick walled elongated sclereids of various shapes and sizes, pitted parenchymatous cells, polygonal cells with beaded walls and several showing thin septum, fibres with peg like growth near tips, lumen broad and criss- cross layer of fibre groups (Haritaki); slightly cuticularised thick-walled and unicellular trichomes, parenchymatous cells containing starch grains, clusters of calcium oxalate crystals and oil globules, stone cells vary from elongated to nearly spherical form with simple pits, parenchymatous cells with reticulate thickening (Bibhitaki); fragments of endosperm cells with starch and crystaloid of aleurone grains, simple rounded and compound starch grains with upto 10 components usually 2-8, individual grains upto 20µ, large feathery, irregular shaped masses of fat, fragments of perisperm cells with schizogenous oil cavities and starch grains in surface view, surface view of testa (Jatīphala); Starch grains, oil globules, cork cells in sectional view, cortical parenchyma cells containing with starch grains & oleoresin cells, thick walled long fibres with oleoresin cells, trichomes and fragments of annular and reticulate vessels (Haldi) (Fig. 2(2A-2F).

Janmaghutti sample-2(J2)

Parenchymatous cells containing oleo-resin, oval to round starch grains not less than 15µ to 30µ and several up to 70µ with hilum ecentric, lamellae distinct; pitted, septate fibres with indentations on its walls (Śunthî); epidermis with papillose and glandular out growths with cuticular striations radiating from base (Ajowan); Thick walled elongated sclereids of various shapes and sizes, pitted parenchymatous cells, polygonal cells with beaded walls and several showing thin septum, fibres with peg like growth near tips, lumen broad and criss- cross layer of fibre groups (Haritaki); slightly cuticularised thick-walled and unicellular Trichomes, Parenchymatous cells containing starch grains, clusters of calcium oxalate crystals and oil globules, Stone cells vary from elongated to nearly spherical form with simple pits, parenchymatous cells with reticulate thickening (Bibhitaki); fragments of endosperm cells with starch and crystaloid of aleurone grains, simple rounded and compound starch grains with upto 10 components usually 2-8, individual grains upto 20µ, large feathery, irregular shaped masses of fat, fragments of perisperm cells with schizogenous oil cavities and starch grains in surface view, surface view of testa (Jatīphala); Starch grains, oil globules, cork cells in sectional view, cortical parenchyma cells containing with starch grains & oleoresin cells, thick walled long fibres with oleoresin cells, trichomes and fragments of annular and reticulate vessels (Haldi): Giant tannin cells, Fragment of epidermis of testa in surface view, Pitted stone cells with thick walls & narrow lumen associated with mesocarp cells (Chuhara); 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); Raphides crystals of calcium oxalate, Endosperm cells in surface

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view embedded with fixed oil globules, aleurone grains, calcium oxalate crystals, Pigments cells of mesotesta in surface view, Sclereids & stone cells from mesocarp (Draksha) ((Fig. 2(2A-2J).

Physico-chemical analysis

The physicochemical parameters such as Loss on drying at 105^0C, extractive values (water soluble extractive and alcohol soluble extractive), ash values (total ash value and acid insoluble ash value), and pH (10 %) aqueous solution were performed in two samples of Janmaghutii (J1 & J2) compound formulations with its used ingredients in triplicate separately. Physico-chemical results are given in (Table 2 & 2a).

Nutritional value analysis

Nutritional value analysis has been performed on two samples of Janmaghutti (J1 & J2) separately in various parameters such as total fat % by weight, Protein (N*5.95) % by weight, Crude fibre % by weight, Total dietary fibre % Carbohydrates by difference, % by weight, Energy value K cals/100mg, Vitamin D -mg/100g, Sodium -mg/100gm, Calcium- mg/100gm, Iron -mg/100g and Potassium- mg/100gm separately. Nutritional value analysis results are given in (Table 3).

Preliminary phytochemical investigation

Qualitative phytochemical tests were performed in water and ethanol extracts. Alkaloids, protein, carbohydrates, flavonoids, resin, and saponins were present in both samples of Janmaghutti (J1 & J2).

Heavy metals tests

Heavy metals (Pb, Cd, As, and Hg) tests were performed and found under limits as prescribed in WHO guidelines, and results are given in (Table 4).

Microbiological limit tests

Microbiological analysis of pathogenic bacteria viz. Salmonella sp., Staphylococcus aureus, Escheria coli, and Pseudomonas aeruginosa were done and found absent in Janmaghutti samples (J1 & J2) where total microbial plate count (TPC) was found 25cfu/g in sample 1 (J1) and 28 cfu/g in sample 2 (J2) and yeast & moulds was found 65 cfu/g in sample1 (J1), 72cfu/g in sample 2 (J2). The microbiological profile of the Janmaghutti two samples (J1 & J2) was found satisfactory under prescribed limits viz. for Salmonella sp., Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus limits absent, where for total microbial plate count (TPC) 10^5cfu/g and for yeast & moulds 10^3cfu/g in WHO/ Ayurvedic Pharmacopoeia of India 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, Chlorfenvinphos mg/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 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, Chlorfenvinphos mg/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 were analyzed in two samples of Janmaghutti (J1 & J2) 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 extracts one spot of the Janmaghutti sample extract with ingredients and Ferulic acid, Kaempferol, and Caffeic acid standard marker spots applied in precoated TLC plate. Applied 6 µl of the test solution as well as a standard marker as 6 mm bands and developed the plate in a solvent system toluene: ethyl acetate (7: 3 v/v) to a distance of 8 cm. dried the developed plate at room temperature and examined. Derivatized the plate using 5% Methanolic-sulphuric acid reagent and dried at 105^0C in a Hot air oven, till the bands were visible. Major spots R_f values with colour were recorded before derivatization at 254nm and after derivatization at 366nm. Major spots of R_f values at 254nm before derivatization are 0.20 black, 0.40 black with Caffeic acid, 0.56 black with Ferulic acid, 0.60 black with Kaempherol standard and after derivatization at 366nm major spots R_f values are 0.20 blue, 0.40 sky blue with Caffiec acid standard, 0.56 sky blue with Ferulic acid standard,0.58 brown with Kaempherol standard, 0.75 sky blue, 0.80 sky blue and 0.90 brown. Chromatograms profile is given (Table 5 & Fig.3, 4 & 5).

Discussion

During the field survey, it was observed that different tribal communities use different formulations of Janmaghutti to maintain the good health of their child, but the basic ingredients are the same. Therefore, after analysis of documented data, we have selected two different types of Janmaghutti formulations (J1 & J2). The J1 sample was prepared with 9 single ingredients while the J2 sample was prepared with 13 ingredients. 9 ingredients are the same for both preparations (J1 & J2), while 4 ingredients are added in sample J2 formulation which is purchased from the market, as mentioned in (Table 1) in preparations of two different formulations of Janmaghutti [25,26, 27].

The Janmaghutti sample 1 (J1) and sample 2 (J2) were standardized as per Ayurvedic Pharmacopoeia of Indian guidelines. The standardization was subjected to various analytical techniques like Organoleptic tests, microscopic tests, physicochemical tests, heavy metals tests, microbiological tests, nutritional value tests, and pesticide tests. Powder microscopic tests were performed and established the distinguished anatomical characters for two different samples (J1 & J2) of Janmaghutti, results are depicted in (Fig 5), and these specific anatomical characters

may be helpful for the identification of ingredients in the Janmaghutti compound formulation[29]. Qualitative phytochemical analysis was performed in water and ethanol extracts. Various phytochemicals like alkaloids, carbohydrates, flavonoids, protein, resin, and saponins were present in both samples (J1 & J2), which could make the formulation useful for potential and preventive healthcare needs. Pesticide residues were analyzed in two samples of Janmaghutti separately and found below the limit of quantification. Similarly, the detection of heavy metals (Pb, Cd, As & Hg) and 42 types of pesticide residue tests were performed and found below limits/absent as per WHO guidelines. Microbiological analysis of pathogenic bacteria, viz. Salmonella sp., Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus were done and found absent in Janmaghutti samples, where total microbial plate count (TPC) was found 25cfu/g in sample 1 (J1) and 28 cfu/g in sample 2 (J2) while yeast & moulds were found 65 cfu/g in sample1 (J1) and 72cfu/g in sample 2 (J2). The microbiological profile of the Janmaghutti two samples (J1 & J2) was found satisfactory under prescribed limits viz. for Salmonella sp., Escheria coli, Pseudomonas aeruginosa, and Staphylococcus aureus limits absent, where for total microbial plate count (TPC) 10^5cfu/g and yeast & moulds 10^3cfu/g given in Ayurvedic Pharmacopoeia of India. Nutritional value analysis of two compound formulations of Janmaghutti samples (JI & J2) were done separately such as total fat was found 11.7%, 16.8%, Protein (N*5.95) 6.7%, 9.4%, Crude fibre 3.4%, 3.2%, Total dietary fibre 16.44%, 14.13%, Carbodydrates 44.2%, 43.0%, Energy value 310 K cals/100mg, 361 K cals/100mg, Vitamin D 0.01 mg/100g, 0.01 mg/100g, Sodium 3601.30 mg/100gm, 3239.30 mg/100gm, Calcium 478.8 mg/100gm, 413.80 mg/100gm, Iron 19.28 mg/100g 16.70 mg/100g, Potassium 1070.87 mg/100gm and 1020.50 mg/100gm respectively. These findings indicate that Janmaghutti (a traditional child health care formulation) is a very good traditional herbal medicine used by the tribal of Chitrakoot region to maintain resistant power and immunity against common ailments of the children.

HPTLC chromatograms of the two samples of Janmaghutti compound formulations with ingredients and with standard marker Ferulic acid, Kaempferol, and Caffeic acid. The R_f values and colour of the resolved bands are noted. Developed chromatograms indicate the presence of Ferulic acid, Kaempferol, and Caffeic acid standards in Janmaghutti samples (J1 & J2). 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. These findings are an indication that Janmaghutti (a traditional herbal child health care compound formulation) is a very good traditional herbal health care drug and nutritious 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 rural as well as urban children. This study may also be helpful in the preparation of Janmaghutti and their quality evaluation.

Conclusion

From the present investigation documentation of traditional medicinal practices and standardization of Janmaghutti, various parameters such as documents of traditional knowledge, selection of formulations, and its importance as a children health care used by tribal of Chitrakoot region. The results of the present study showed satisfaction with the efficacy and potency of the products to maintain resistant power and immunity against common ailments of the children to attain the life-long health goal. The developed traditional formulation affords the health care needs in society. In the future, this data may be used as a standard for the production

of quality formulation and standardized finished product of Janmaghutti It also serves as a reference monograph in the preparation of children's health care traditional drugs.

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Ethical issues

There is none to be applied

Conflict of interest

None

References

1 Anonymous 1999. an appraisal of trible-folk medicines, CCRAS,1st ed., New Delhi,1999.
2 Mutakar RK, Arati Kaulgekar 2004. a survey report, interdisciplinary school of health sciences, university of Pune, India,
3 Chaturvedi A, Tiwari A K, Mani R J. 2017. Traditional practices of using various medicinal plants during postnatal care in Chitrakoot district, Indian J Tradit Know 16(4): 605-613.
4 Rajith N P, Navas M, Thaha A M, Manju M J, Anish N, Rajasekharan S, George V. 2010. A Study on traditional mother care plants of rural communities of South Kerala, Indian J Tradit Know 9 (1): 203-208.
5 Chaturvedi A. 2011. Documentation of traditional medicinal knowledge of Chitrakoot region, Technical report, (Government of India, Council of Scientific & Industrial Research, New Delhi).
6 Nergard C S, Than Ho T P, Diallo D, Ballo N, Paulsen BS, Nordeng H. 2015. Attitudes and use of medicinal plants during pregnancy among women at health care centres in three regions of Mali, West Africa. J Ethnobiol Ethnomed 11:73.
7 Fabricant D S, Farnsworth N R. 2001. The value of plants used in traditional medicine for drug discovery, Environ. Health Protect, 109 Suppl 1: 69-75.
8 Kokate C K, Purohit A P, Gokhile SB. 2005. Pharmacognosy, Nirala Prakashan, 30th Edn.
9 Evans WC. 2003. Treatise & Evans, Pharmacognosy, Saunders, London,15th ed. pp 545-547.
10 Kokate CK. 2006. Practical Pharmacognosy, Vallabh Prakashan, New Delhi.
11 Anonymous 2009. Laboratory guideline for the analysis of Ayurveda and Siddha formulations, published by Central Council for Research in Ayurveda and Siddha Deptt. Of AYUSH, Ministry of Health & Family Welfare Government of India, Janakpuri, New Delhi.
12 Anonymous 1991. Guidelines for the assessment of herbal medicines document no. WHO/TRM, 91, 4 World Health Organizations, Geneva.
13 IS: 14433 2007. Indian Standards (IS), infant milk substitute-specification (first revision) Bureau of Indian Standards, Manak Bhavan, 9 Bahadur Shah Zafar Marg, New Delhi,
14 Anonymous 2016. Manual of Method of Analysis of Foods, Food Safety and Standards Authority of India (FSSAI) Ministry of Health and family welfare, government of India New Delhi.
15 Venugopal G, Srinivasa R C, Rao N B, Rao V B, Rao V T, Rao A P. 2015. Pharmacognostic and Preliminary Phytochemical Evaluation of Ocimum basilicum L. var. pilosum (Willd.) Benth.

and O. tenuiflorum var. CIM-AYU, International Journal of Pharmacognosy and Phytochemical Research **7**(3): 519-526.
16 Tripathi M, Sikarwar R L S. 2014. Pharmacognostic Standardization of stem bark of Erythrina variegata L. Journal of the Indian Botanical Society 93 (3&4): 248-253.
17 Anonymous 2007. Protocol for Testing of Ayurvedic, Siddha & Unani Medicines, Ministry of Health & Family Welfare, Pharmacopoeial Laboratory for Indian Medicines, Ghaziabad.
18 Tiwari A, Tripathi M, Dwivedi N. 2015. Standardization of Ayurvedic compound formulation Kutajastaka Kvatha Curna J. Trop. Med. Plants 15: 1-13.
19 Anonymous 2010. Laboratory guide for the analysis of Ayurveda and Siddha formulations, published by Central Council for Research in Ayurveda and Siddha, Department of AYUSH, Ministry of Health and Family Welfare, Government of India, New Delhi, 2010, 83-87.
20 Anonymous 2008. Quality Control Manual for Ayurvedic, Siddha & Unani Medicine published by Government of India, Department of AYUSH, Ministry of Health & Family Welfare, Pharmacopoeial Laboratory for Indian Medicine, Ghaziabad.
21 Choudhary N, Siddiqui M B, Khatoon S. 2014. Pharmacognostic evaluation of Tinospora cordifolia Willd.) Miers and identification of biomarkers, Indian J Tradit Know 13(3): 543-540.
22 Sikarwar R L S, Tripathi M, Pathak B. 2012. Ethnomedicinal plants sold by herbal vendors in the Kamadgiri Parikrima Marg in Chitrakoot (M.P.). Ethnobotany 24 (1&2): 114-118.
23 Tripathi I P, Dwivedi N. 2013. Standardization of Janamghutti, A Folk-loric Herbal Formulation of Tribals of Chitrakoot region, District Satna, Madhya Pradesh, India, Int. J. Res. Ayurveda Pharm 4(3): 135-138.
24 Dwivedi N, Tripathi M, Tripathi I P. 2012. Documentation of Traditional Medicinal knowledge at Chitrakoot region, Distt. Satna (M.P.) with special reference to Janamghutti. Life Science Bulletin 9(1): 139-141.
25 Tripathi M, Sikarwar R L S. 2013. Some Traditional Herbal Formulations of Chitrakoot region, Madhya Pradesh, India, Indian J Tradit Know 12(2): 315-320.
26 Tripathi M, Sikarwar RLS, Singh R. 2019. Documentation of indigenous knowledge through seminar of traditional medicine men. Ethnobotany 30: 123-132.
27 Tripathi M, Sikarwar R L S, Tiwari A, Dwivedi N. 2015. Pharmacognostical identification of ingredients in Laghulai curna, Indian J Tradit Know 14(4): 531-536.

Table 1- Formulation Composition of Janmaghutti

S. No	**Formulations name** **Janmaghutti sample 1 (J1)**	**Botanical name**	**Part used**	**Quantity**
1	Śunthî	Zingiber officinale Rosc. (Zingiberaceae)	Rhizome	1part
2	Ajowan	Trachyspermum ammi (L.) Sprague (Apiaceae)	Fruit	1part
3	Haritaki	Terminalia chebula Retz. (Combretaceae)	Fruit	1part
4	Bibhitaki	Terminalia bellirica (Gaertn.)	Fruit	1part

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		Roxb. (Combretaceae)		
5	Jatīphala	Myristica fragrans Houtt. (Myristicaceae)	Seed	1part
6	Haldi	Curcuma longa L. (Zingiberaceae)	Rhizome	1part
7	Rãmatha (Hingu)	Ferula assa-foetida L. (Apiaceae)	Exd.	1part
8	Sauvarcala	Black salt	-	1part
9	Saindhava	Rock salt		1part
10	Mother milk	-	-	
	Janmaghutti sample 2 (J2)			
1	Śunthî	Zingiber officinale Rosc. (Zingiberaceae)	Rhizome	1part
2	Ajowan	Trachyspermum ammi (L.) Sprague (Apiaceae)	Fruit	1part
3	Haritaki	Terminalia chebula Retz. (Combretaceae)	Fruit	1part
4	Bibhitaki	Terminalia bellirica (Gaertn.) Roxb. (Combretaceae)	Fruit	1part
5	Jatīphala	Myristica fragrans Houtt. (Myristicaceae)	Fruit	1part
6	Haldi	Curcuma longa L. (Zingiberaceae)	Phizome	1part
7	Rãmatha (Hingu)	Ferula asaa-foetida L. (Apiaceae)	Exd.	1/2part
8	Sauvarcala	Black salt	-	1/2part
9	Saindhava	Rock salt		1/2part
10	Kaju	Anacardium occidentale L. (Anacardiaceae)	Fruit	1/2part
11	Badam	Prunus amygdalus Batsch (Rosaceae)	Fruit	1/4part
12	Chohara	Phoenix dactylifera L. (Arecaceae)	Fruit	1/4part
13	Draksha	Vitis vinifera L. (Vitaceae)	Fruit	1part
14	Mother milk	-	-	-

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Table 2- Physico-chemical parameters of Janmaghutti samples and used single drugs

Name of samples/ingredients	LOD (% w/w)	Mean (%)	Total ash (% w/w)	Mean (%)	AI ash (% w/w)	Mean (%)	ASE (% w/w)	Mean (%)	WSE (% w/w)	Mean (%)
Janmaghutti Sample1 (J1)	8.5 8.4 8.6	8.5	25.3 25.2 25.4	25.3	5.30 5.25 5.35	5.3	22.33 22.81 22.48	22.54	47.6 47.9 47.3	47.6
Janmaghutti Sample2 (J2)	7. 9 8.0 7.8	7.9	19.6 19.7 19.5	19.6	4.60 4.15 4.20	4.31	21.35 21.15 21.65	21.38	48.20 48.56 48.59	48.45
Śunthî	6.92 6.95 6.98	6.95	6.00 6.01 6.00	6.00	0.99 0.98 0.94	0.97	6.10 6.15 6.17	6.14	15.46 15.41 15.50	15.46
Ajowan	8.40 8.48 8.60	8.49	5.61 5.44 5.64	5.56	0.19 0.11 0.12	0.14	4.16 4.20 4.32	4.22	18.76 18.40 19.20	18.78
Haritaki	5.18 5.44 5.42	5.3	3.35 3.24 3.19	3.20	2.10 2.02 2.15	2.09	67.25 62.35 70.50	66.70	51.40 51.20 51.40	51.33
Bibhitaka	6.42 6.21 6.47	6.3	4.08 4.04 4.12	4.08	2.99 2.98 2.94	2.97	49.45 50.05 49.85	49.78	57.95 56.55 57.10	57.20
Jatiphala	7.87 8.82 8.92	8.53	2.65 2.45 2.42	2.50	0.42 0.44 0.36	0.40	14.42 13.65 14.21	14.09	9.86 10.21 9.91	9.91
Haridra	5.18 5.32 5.58	5.36	5.42 5.10 5.30	5.27	1.26 1.38 1.20	1.28	13.48 13.60 13.20	13.42	14.70 14.19 14.42	14.43
Hingu (Exd.)	5.34 5.83 5.98	5.71	8.00 8.24 8.40	8.21	1.90 1.80 1.80	1.85	61.81 60.92 61.20	61.31	64.20 64.40 63.40	64
Kaju	7.80 7.59 7.74	7.71	9.6 9.95 9.8	9.78	1.6 1.5 1.75	1.61	35.45 35.50 35.65	35.53	54.3 54.65 54.80	54.58
Badam	8.10 8.15 8.14	8.13	10.0 10.20 10.15	10.11	2.34 2.50 2.65	2.49	38.40 38.30 38.25	38.31	52.2 52.1 52.3	52.2
Chuhara	11.5 11.6 11.4	11.5	5.3 5.4 5.6	5.43	1.3 1.2 1.4	1.3	23.45 23. 50 23.40	23.45	65.75 65.35 65.40	65.5
Draksha	12.35 12.60 12.25	12.4	6.35 6.55 6.70	6.53	1.2 1.4 1.5	1.36	27.8 27.6 27.5	27.63	70.0 70.4 70.3	70.23

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Table 2a- Physico-chemical analysis of Lavana (salts) single drugs

Type of salt	Physical property	Effect of Heat	Solubility	Assay
Sauvarcala (Black salt)	Crystalline coarse-grained aggregates, black-coloured, salty taste	On burning gives yellow sodium fumes. When heated through a blowpipe, produces a blue flame of chlorine	soluble in water	Sodium- 1711.0 ppm Potassium - 51.6 ppm
Saindhava (Rock salt)	Crystalline coarse-grained aggregates, off-white coloured, salty taste	On burning gives yellow sodium fumes. When heated through a blowpipe, produces a blue flame of chlorine	soluble in water	Sodium-1629.0 ppm Potassium-253.3 ppm

Table 3 -Nutritional Value Analysis of Janmaghutti

S. No	Parameters	Unit of measurement	Sample 1 (J1)	Sample 2 (J2)
1	Total fat	% by wt.	11.7%	16.8%
2	Protein (N*5.95)	% by wt.	6.9%	9.4%
3	Crude fibre,	% by wt.	3.4%	3.2%
4	Total Dietary fibre,	%	16.44%	14.13%
5	Carbohydrates	by difference, % by wt.	44.2%	43.0%
6	Energy value	K cals/100mg	310	361
7	Vitamin D	mg/100g	0.01	0.01
8	Sodium (Na)	mg/100g	3601.30	3239.30
9	Calcium (Ca)	mg/100gm	478.80	413.80
10	Iron (Fe)	mg/100g	19.28	16.70
11	Potassium (K)	mg/100g	1070.87	1020.50

Table 4- Determination of Heavy Metals in Janmaghutti

S. N.	Parameters	Sample 1 (J1)	Sample 2 (J2)	API Limits
1	Lead (Pb)	0.4103ppm	0.8173ppm	10 ppm
2	Cadmium (Cd)	0.0529ppm	0.0596ppm	0.3 ppm
3	Arsenic (As)	0.5346ppb	0.6128ppb	03 ppm
4	Mercury (Hg)	0.6156ppb	0.5432ppb	01 ppm

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Table 5- Calibration parameters of marker compounds

Parameter	Ferulic acid	Kaempferol	Caffeic acid
Linearity range (µg/spot)	200-600	200-600	200-600
R_f	0.516	0.578	0.381
Regression Equation	y = 2E-05x + 0.008	y = 3E-05x - 0.000	y = 1E-05x + 0.000
Correlation coefficient	0.996	0.995	0.999
Regression coefficient (R^2)	0.993	0.991	0.999
Average	0.0182	0.0101	0.0051
Slope	0.00002	0.00002	0.00001
Standard deviation	0.00469	0.00549	0.00218
Standard error	0.00055	0.00077	9.38971E-05
Intercept	0.0089	-0.00087	0.0007

Fig.1-Documentation of traditional knowledge (A, B), Preparation of Janmaghutti (C), Administration of Janmaghutti (D)

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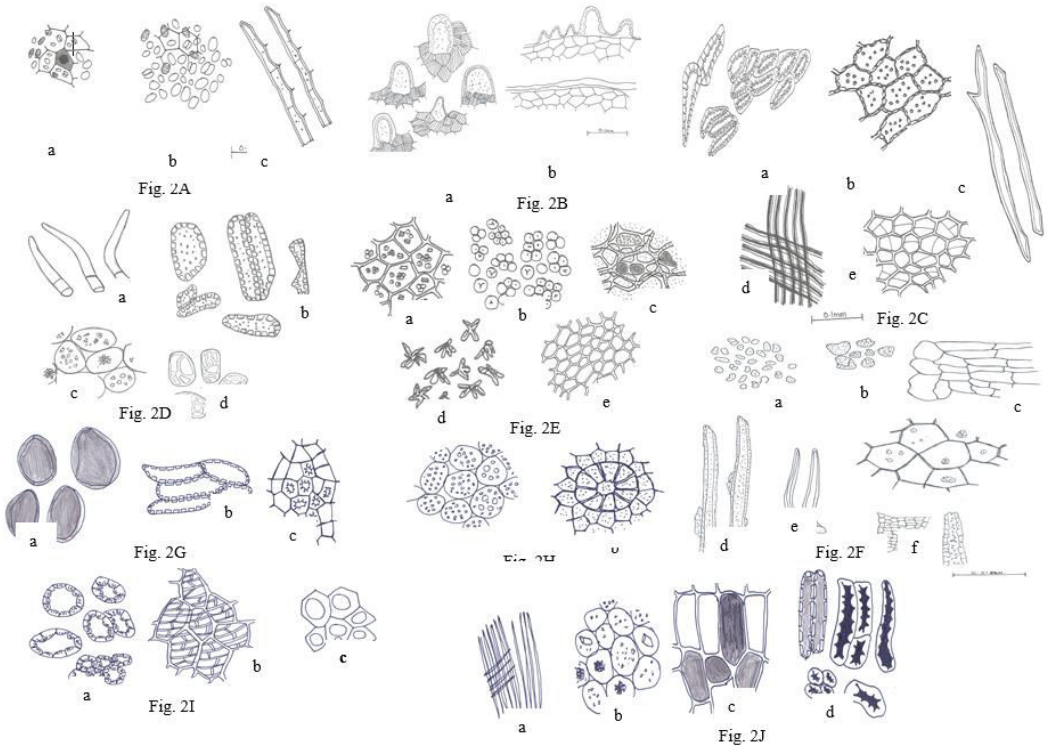

Fig.2- Powder microscopy of Janmaghutti (2A) Sunthi (a) Parenchymatous cells containing oleo-resin (b) oval to round starch grains not less than 15µ to 30µ and several up to 70µ with hilum ecentric, lamellae distinct (c) pitted, septate fibres with indentations on its walls (2B) Ajowan (a) glandular trichomes (b) epidermis with papillose and glandular out growths with cuticular striations radiating from base (2C) Haritaki (a) Thick walled elongated sclereids of various shapes and sizes (b) pitted parenchymatous cells (c) polygonal cells with beaded walls and several showing thin septum (d) fibres with peg like growth near tips (e) lumen broad and criss- cross layer of fibre groups (2D) Bibhitaki (a) slightly cuticularised thick-walled and unicellular trichomes (b) Parenchymatous cells containing starch grains, clusters of calcium oxalate crystals and oil globules (c) Stone cells vary from elongated to nearly spherical form with simple pits (d) parenchymatous cells with reticulate thickening (2E) Jatiphala (a) fragments of endosperm cells with starch and crystaloid of aleurone grains (b) simple rounded and compound starch grains with upto 10 components usually 2-8, individual grains upto 20µ (c) large feathery, irregular shaped masses of fat (d) fragments of perisperm cells with schizogenous oil cavities and starch grains in surface view (e) surface view of testa (2F) Haldi (a) Starch grains (b) oil globules (c) cork cells in sectional view (d) cortical parenchyma cells containing with starch grains & oleoresin cells (e) thick walled long fibres with oleoresin cells (f) trichomes (g) fragments of annular and reticulate vessels (2G) Chuhara (a) Giant tannin cells (b) Fragment of epidermis of testa in surface view (c) Pitted stone cells with thick walls & narrow lumen associated with mesocarp cells (2H) Kaju (a) Mesophyll cells in surfaces view filled with starch grains, aleurone grains & oil globules (b) Oil globules embedded within mesophyll cells (2I) Badam (a) Stone cells (b) Reticulate parenchyma (c) endosperm cells filled with aleurone grains & oil globules (2J) Draksha (a) Raphides crystals of calcium oxalate (b) Endosperm cells in surface view embedded with fixed oil globules (c) aleurone grains (d) calcium oxalate crystals (e) Pigments cells of mesotesta in surface view (f) Sclereids & stone cells from mesocarp

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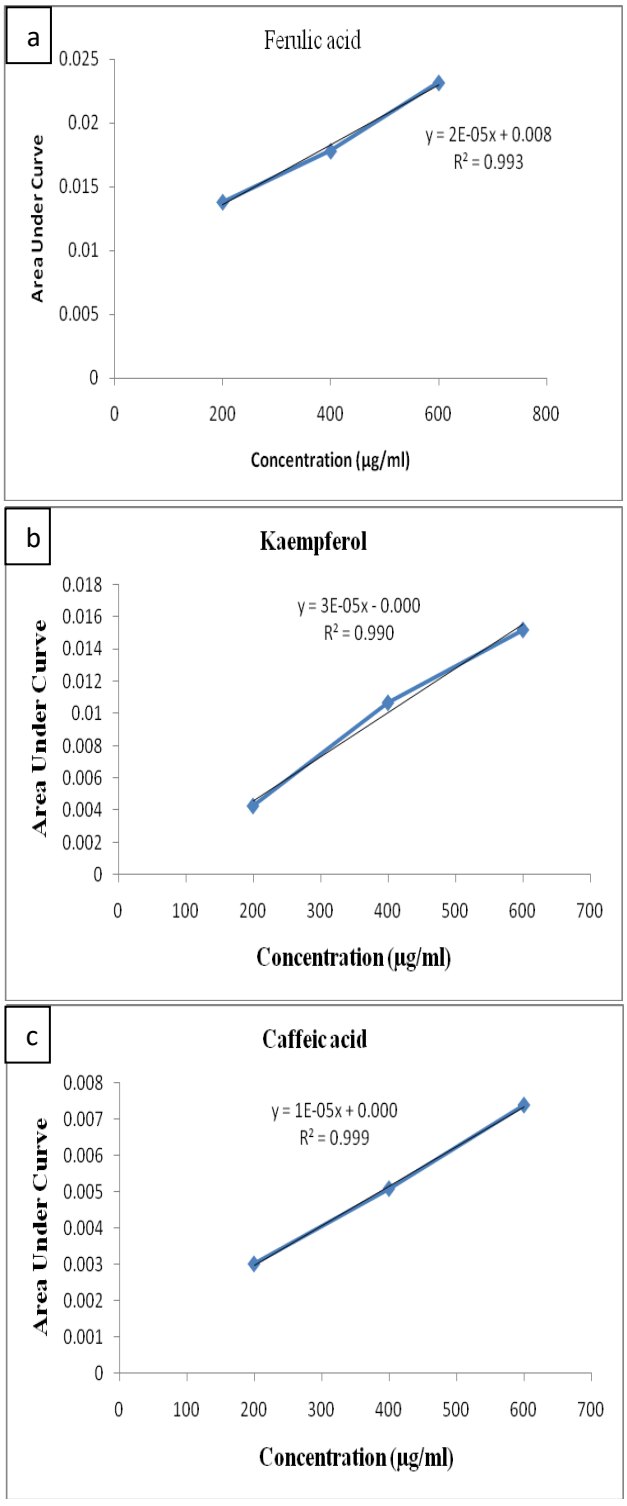

Fig. 3 - Calibration curve of marker compounds (a) Ferulic acid, (b) Kaempferol and (c) Caffeic acid

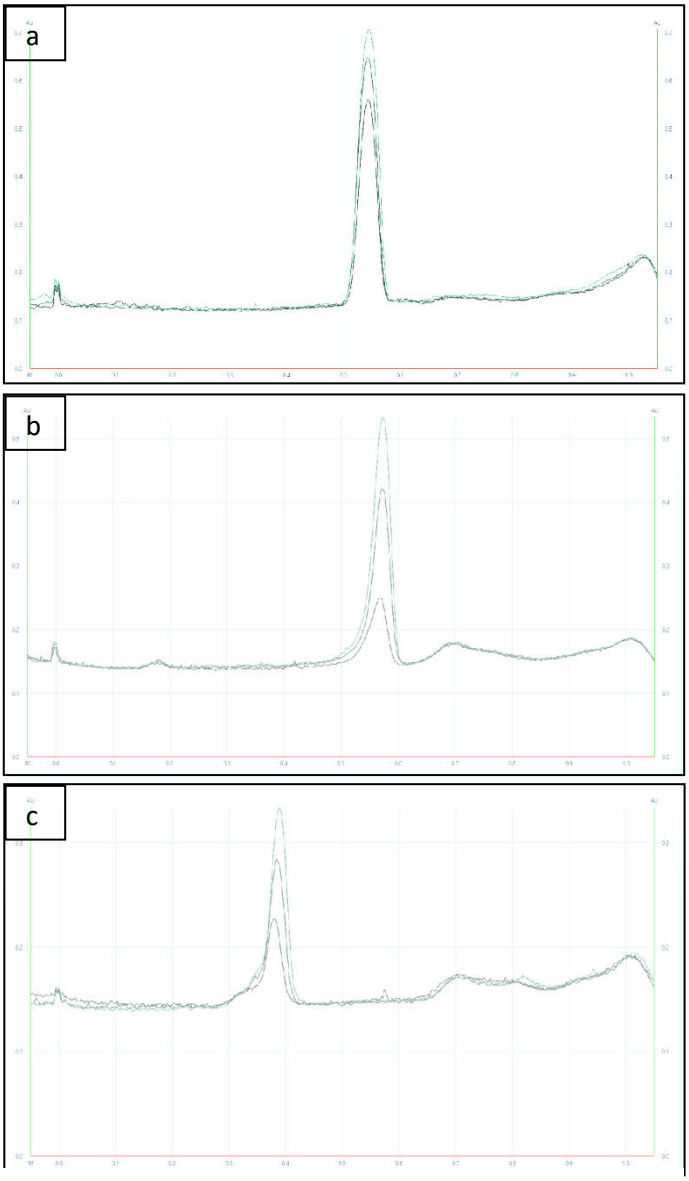

Fig. 4- Purity spectra of marker compounds (a) Ferulic acid (b) Kaempferol and (c) Caffeic acid

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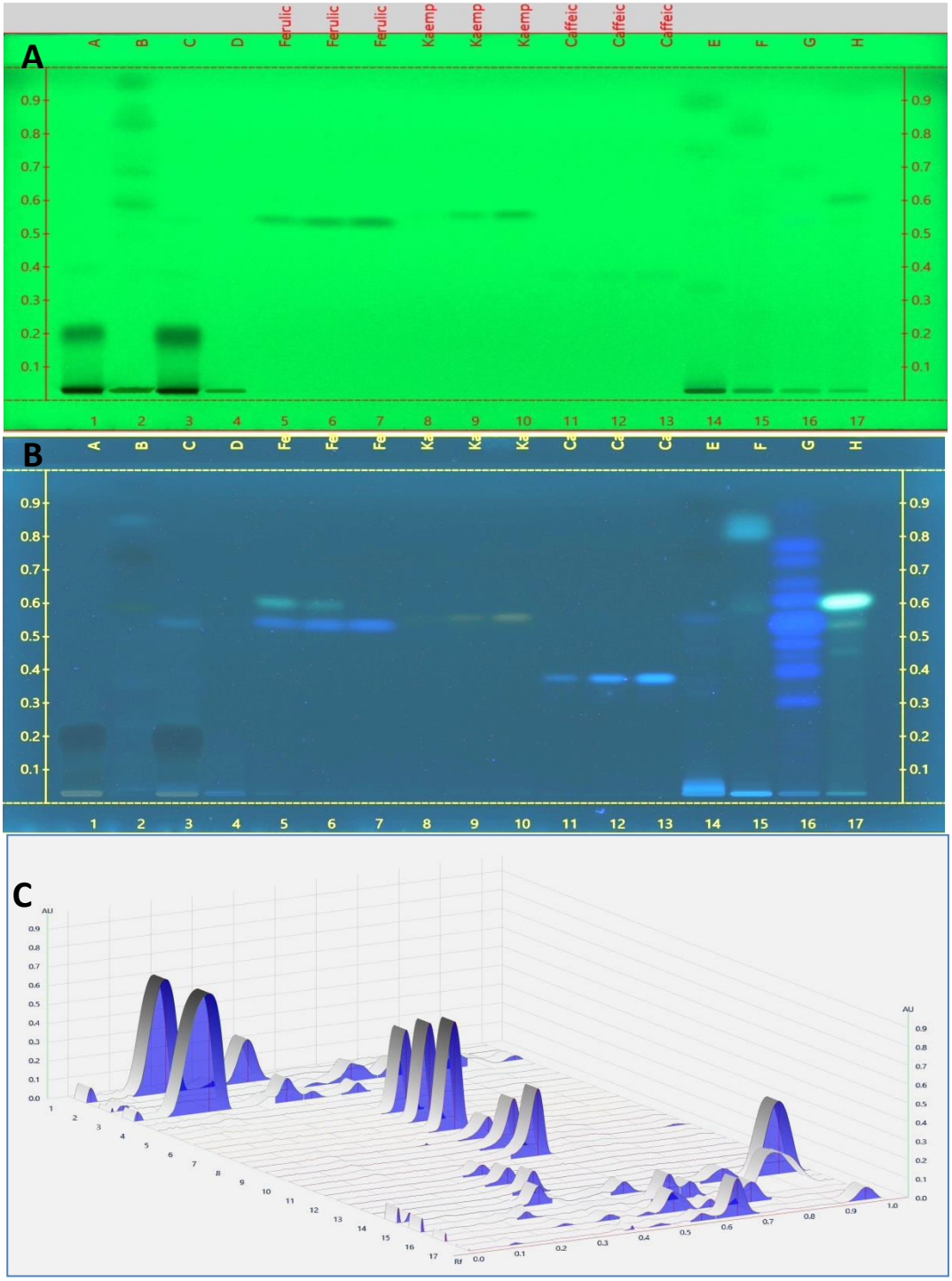

Fig.5- HPTLC chromatograph of the *Janamghutti* at (A) at 254nm before derivatization, (B) at 366nm after derivatization, (C) 3D densitometry graph

Abbreviations- track1 sunthi , track 2 Janmaghutti(J1), track 3 ajowan, track 4 hingu, track 5-7 ferulic acid, track 8-10 kempherol, track 11-13 caffeic acid, track 14 haritaki, track 15 bibhitaki, track 16 Jatiphala, track 17 haldi