



## FORMULATION AND PHARMACOGNOSTICAL INVESTIGATION OF FOLKLORE PRACTICES WITH SPECIAL REFERENCE TO ANAMEIA CONTROL AT CHITRAKOOT REGION OF MADHYA PRADESH

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### INTRODUCTION

Traditional practices is defined by the World Health Organization (WHO) as "single or combined application of manual techniques and exercises, spiritual therapies, plant, animal, and mineral-based practices and knowledge and beliefs incorporating to treat, diagnose, and prevent illnesses or maintain well-being. Chitrakoot region is a remote area situated in the northern region of Satna district of Madhya Pradesh. The tribal of the region have well developed folklore practices used to maintain the basic health care need. It is well known that plant are the great source of bioactive compounds having tremendous medicinal properties and can be used to discover plant based drugs with lesser side effect. A survey was carried out among the tribal community of Chitrakoot region, Satna district of Madhya Pradesh to explore the folklore practices interviewed among the local healers and elderly women. A total 674 interview conducted in 86 Intensive field visits in 18 tribal dominated villages were carried out during 2020-2021, covering almost all the seasons of the year. The 45-80 years old knowledgeable men and women, experienced traditional healers and local vaidyas who have actively engaged in ethno-medicinal practices, were interviewed. For pharmacognostical standardization various parameters are involved including macroscopy, powder microscopy, physicochemical tests, nutritional value analysis phytochemical analysis, high performance thin layer chromatography (HPTLC) fingerprints profile were carried out. Macroscopic and microscopic studies were done and distinguish characters of formulations with ingredients were established. Physicochemical tests were done and results obtained separately. Similarly detection of heavy metals (Pb, Cd, As & Hg) were performed and found under limits/absent as per WHO guidelines. Nutritional value analysis of two compound formulations (ACF1 & ACF2) were done such as total fat % by wt. 17.40%, 19.94%, Protein (N\*5.95) % by wt. 10.75%, 12.87%, Total dietary fibre % 3.90%, 4.89 %, Carbohydrates by difference, % by wt. 45.60%, 57.18%, Energy value K cal/100mg 395.10, 459.66, Sodium mg/100gm 62.34, 72.50, Calcium mg/100gm 12.50, 14.00, Iron mg/100g 8.60, 10.43, Potassium mg/100gm 105.60 and 140.80 were found respectively. These findings indicate that formulations (ACF1 & ACF2) are beneficial used by tribal of Chitrakoot region to maintain resistant power, immunity and against anaemia common illness. And it can be used as strength promoter, immuno modulator

and anaemia controller. These folklore practices if integrated with modern healthcare system could elevate the health status of the rural as well as urban people.

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**Keywords:** Folklore practices, HPTLC fingerprinting, Formulations, Nutritional value, Pharmacognostic

## INTRODUCTION

Anaemia is a most common human disease of the India as well as the world. It is defined as decreased concentration of haemoglobin in blood or less number of red blood cells in blood or their insufficient oxygen carrying to body's as per physical requirements WHO, (2020). Anaemia is higher among developing countries than the developed countries due to low socioeconomic status and indigent access to healthcare services Sasidharannair *et al* (2019). It is estimated that 2.36 billion people anaemia affected individuals globally especially women and children Stevens *et al* (2013). The prevalence of anaemia is estimated to be higher in India when compared to all other developing countries Ramachandran & Kalaivani (2018). Also, it is the second leading cause of maternal deaths in the country Vindhya *et al* (2019). 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 area who are untouched by modern civilization use plants for their basic health care.

Chitrakoot is one such area situated in the northern region of Satna District of Madhya Pradesh, India. It lies between 80<sup>0</sup>52' to 80<sup>0</sup>73' in latitude and 25<sup>0</sup>10' to 25<sup>0</sup>52' longitude, covering an area of 1584sq km. It is inhabited by various tribal communities like Mawasi, Khairwar, Kol, and Gond. There are a lot of traditional societies in rural areas of Chitrakoot region 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 (Nergard *et.al*. 2015). The young generation due to urbanization and fast changing trends in their life style does not want to follow the footsteps of their forefathers. As a consequence this knowledge is getting eroded. These rich traditions of India that continue to provide health care needs of the vast majority of rural masses need to be protected before it is lost forever. In this concern, the work has been undertaken to document folklore practices with special reference to anaemia control formulations used in almost all the household all over India for centuries to prevent the anaemia and for strengthening the immune system of the human. As a general rule in assessment of traditional medicine, traditional experience means that long term use as well as the medical, historical and ethnobiological background of these medicines should be taken into account. A considerably researches have supported ethnobiological background of the traditional medicines. Various workers tried to do systematic documentation on use of traditional medicines from different region of country (Fabricant &, Farnsworth 2001). Therefore, it is important for developing better pharmacognostic standardization techniques for folklore practices and so for quality control standards of the plants used in indigenous system of medicine is becoming more relevant today. For pharmacognostic standardization of the formulations the work deals with anatomical evaluation, physicochemical tests, preliminary phytochemical screening, heavy metals test, High Performance Thin Layer Chromatography and nutritional value analysis.

## METHODOLOGY

### Systematic survey and documentation of folk-lore/traditional formulations

For present study a systematic survey and documentation of traditional knowledge used for malnutrition and anaemia control in Chitrakoot region, Satna (M.P.) by rural people of Chitrakoot region. 18 tribal dominant villages surveyed, these are Amiliya, Barua, Bhargawan, Bundelapur, Chandai, Devlaha, Tagi, Hiraundi, Kailashpur, Kathauta, Koldari, Parewa, Patna, Patni, Pindra, Piparitola, Raiya and Turra. 674 interview conducted in 86 Intensive field visits were carried out during 2020-2021, covering almost all the seasons of the year, The 45-80 years old knowledgeable men and women, experienced traditional healers and local vaidyas who have actively engaged in ethno-medicinal practices, were interviewed. The detailed information regarding preparation and uses of the formulation such as plants (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 same localities and in the other localities.

### Collection of samples and formulations preparation

The fresh ingredients of the formulations (ACF1 & ACF2) were collected from forest Satawar (*Asparagus racemosus* Willd family- Asparagaceae) Rhizome, Ashwagandha (*Withania somnifera* (Linn.) Dunal family- Solanaceae) root, Safed Musali (*Chlorophytum borivalianum* (Roxb.) family - Liliaceae) root, Mahua (*Madhuca longifolia* (Koen.) family- Sapotaceae) flowers, Babool exude (*Vachellia nilotica* Linn., family- Mimosaceae ) and also purchased from Chitrakoot market Dist. Satna, Madhya Pradesh Til (*Sesamum indicum* Linn. Family- Pedaliaceae) seeds, Samples were identified and authenticated as per standard procedure (Pushpagandan & George, 2010) by Dr. Manoj Tripathi Senior Scientist, Deendayal Research Institute Chitrakoot. Fresh material was used for anatomical studies whereas shade dried material was powdered in electric grinder for further studies. Two different types of formulations (ACF1 & ACF2) were prepared separately by using 6 ingredients.

### Method for preparation of Anaemia control formulation sample (ACF1)

For preparation of the Anaemia control formulation sample (ACF1) ingredients (Satawar Rhizome, Ashwagandha root and Safed Musali root) were taken in specific quantity. Clean, wash, dry and powdered the samples separately. Weighed each ingredients separately and mix together in specific quantity and enough to pass through 355  $\mu\text{m}$  IS Sieve (old sieve number 44) to obtain a homogenous blend. Stored it in air-tight containers for further analysis.

### Method for preparation of Anaemia control formulation sample (ACF2)

Similarly Anaemia control formulation sample (ACF2) is prepared by 3 ingredients (*viz.* Mahua flowers, Babool exude and Til seeds) were taken separately in specific quantity. Clean, wash, dry and powdered the samples separately. Weighed each ingredients separately and mix together in specific quantity and enough to pass through 355  $\mu\text{m}$  IS Sieve (old sieve number 44) to obtain a homogenous blend. Stored it in air-tight containers for further analysis.

### Macroscopic study

Organoleptic characters or macroscopic of Anaemia control formulation samples (ACF1 & ACF2) samples like colour, odour and taste were evaluated.

### **Powder microscopic study of Anaemia control formulation samples (ACF1 &ACF2)**

About 50 gm of powder each samples (ACF1 &ACF2) took on a 106  $\mu$ m IS Sieve used lukewarm water from a wash bottle sprayed a jet on to 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. Repeated the process for 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 microscope at 40 x 10x magnification of the Trinocular Research Microscope separately (Jain SK, 2004 and Kokate et.al.2005).

### **Physico-chemical tests**

Physico-chemical parameters such as moisture content (loss on drying at 105<sup>0</sup>C), water soluble extractive value, alcohol soluble extractive value, total ash value, acid insoluble ash value and *pH* (10 %) aqueous solution were Performed two samples of Anaemia control formulation samples (ACF1 &ACF2) (Evans W C, Trease & Evans 2003 and Kokate CK,2006).

### **Nutritional Value Analysis**

Nutritional value analysis have been performed two samples of Anaemia control formulation samples (ACF1 &ACF2)) separately in various parameters such as such as total fat % by weight, Protein (N\*5.95) % by weight, Total dietary fibre % Carbohydrates by difference, % by weight, Energy value K cal/100mg, Vitamin D -mg/100g, Sodium -mg/100gm, Calcium-mg/100gm, Iron -mg/100g and Potassium- mg/100gm as per standard methods (Anonymous 2009 and IS:14433, 2007).

### **Screening of heavy metals tests**

Heavy metals are toxic and generally occur through earth in plants. Mainly four types of heavy metals harmful for us they are Pb, Cd, As and Hg. These heavy metals detected through Atomic Absorption Spectrophotometer as per standard method (Anonymous 1991).

### **Preliminary phyto-chemical analysis**

Preliminary phyto-chemical analysis of two samples of Anaemia control formulation samples (ACF1 &ACF2)) were performed on ethanolic extract and water extract for the presence\absence of phyto-constituents like alkaloids, carbohydrates, flavonoids, protein, resins, saponin, tannin and steroids separately (Venugopal et al.2015 and Anonymous 2016).

### **High performance thin layer chromatography (HPTLC) fingerprint profile**

For High performance thin layer chromatography, 5 gm of each samples Anaemia control formulation samples (ACF1 &ACF2) powder were extracted with 100 ml of methanol overnight, filtered and concentrated. High performance thin layer chromatography (HPTLC) study of the methanolic extracts one spot of the formulation sample extract with ingredients spots applied in precoated TLC plate. Applied 6  $\mu$ l of the test solution 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 in room temperature and examined. Derivatized the plate using 5% *Methanolic-sulphuric acid* reagent and dry at 105<sup>0</sup>C in Hot air oven, till the bands were

clearly visible. Major spots  $R_f$  values with colour were recorded before derivatization at 366nm and after derivatization at visible light and at 366nm (Tripathi & Sikarwar2014 and Anonymous, 2007).

## RESULTS & DISCUSSION

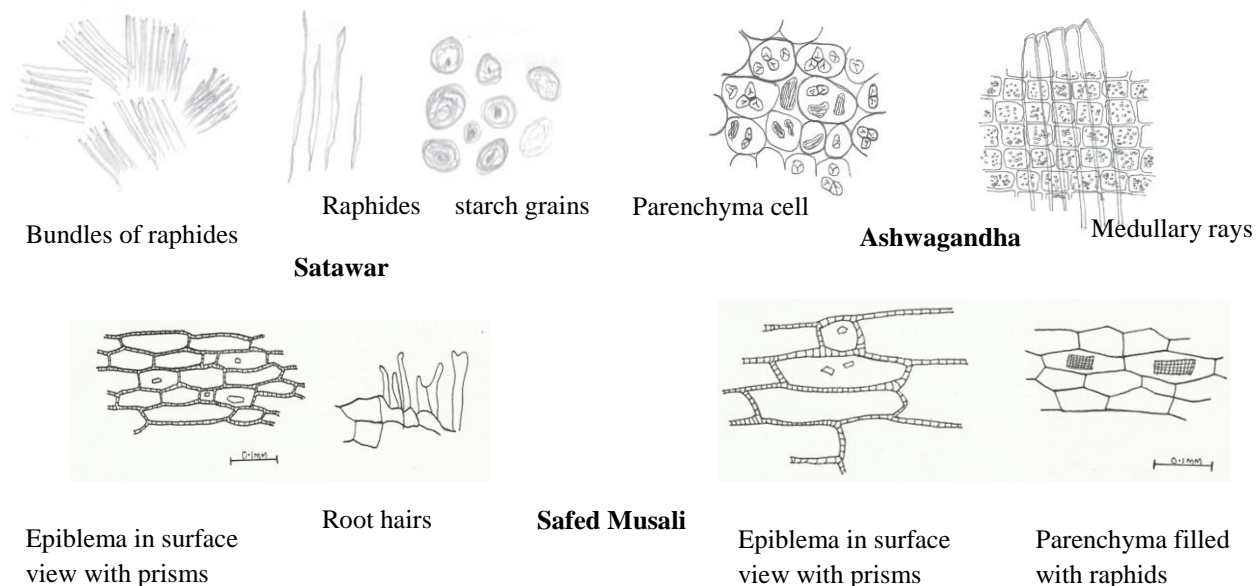
### Macroscopic characters

A brownish white powder color, with the characteristic odour of and tasteless taste of Anaemia control formulation (ACF1). And Anaemia control formulation (ACF2)- color blackish brown granulated Powder/Cake/Laddu with the characteristic odour of Madhuca and a light sweetish taste.

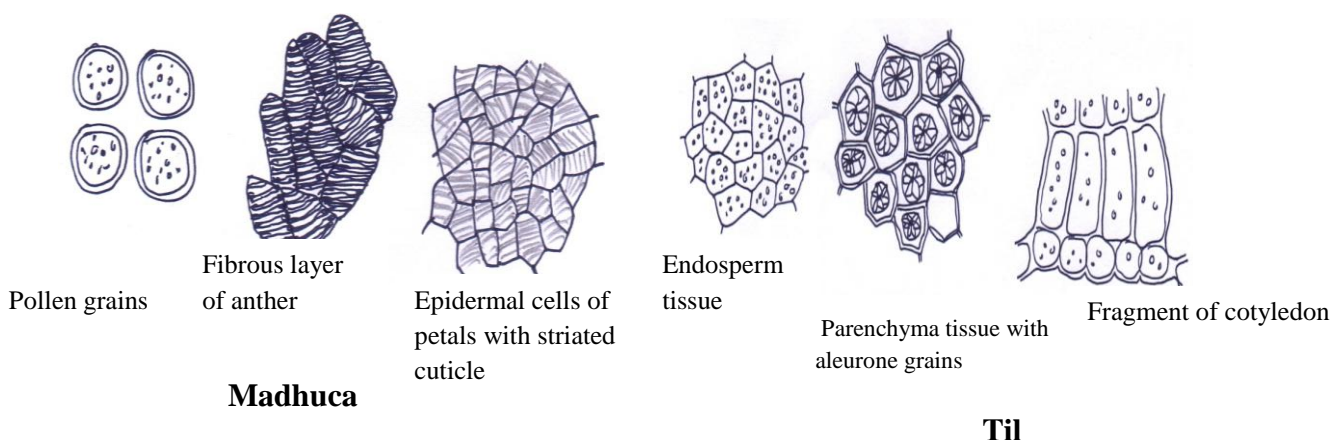
### Powder microscopic characters

**Anaemia control formulation sample (ACF1):** Under microscopic observation following distinguished anatomical characters were identified Bundles of raphids, long spindle shaped raphids and long oval in various shaped found starch grains (Satawar); cortical parenchymatous cells and medullary rays cells containing simple starch grains that are up to  $30\mu$ , mular shaped, mostly single, occasionally groups of two or three with slit like or stellate hilum (Ashwagandha) and 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 (Safed Musali) (Plate1).

**Anaemia control formulation sample (ACF2): Mahua flower-** Pollen grains, fibrous layer of anther, epidermal cells of petals with striated cuticle, covering trichomes, fragments of pigment vessels, glandular trichomes; **Til seeds-** fragment of endosperm tissue in surface view embedded with aleurone grains and oil globules, parenchyma tissue with aleurone grains, fragment of cotyledon and **Babool exude-** It is a natural gum got from the Babool plant. Gum is a dietary fibre and dissolved in water. Gum color is pinkish brown, fibrous, smooth, taste sweet and astringent odor (Plate2).



**Plate 1: Powder microscopic characters of Anaemia control formulation (ACF1)**



## Plate-2: Powder microscopic characters of Anaemia control formulation (ACF2)

### Physico-chemical analysis

The physico-chemical analysis such as Loss on drying on  $105^{\circ}\text{C}$ , water such as extractive values, alcohol soluble extractive value, total ash value and acid insoluble ash value were performed. The results are expressed as mean ( $n=3$ )  $\pm$  standard deviation in w/w. Anaemia control formulation (ACF1) LOD was found 6.59% w/w, total ash value 9.50% w/w, acid insoluble ash value 1.34% w/w, alcohol soluble extractive value 51.63% w/w and water soluble extractive value 49.16% w/w. While Anaemia control formulation (ACF2) LOD was found 5.00% w/w, total ash value 8.64% w/w, acid insoluble ash value 1.23% w/w, alcohol soluble extractive value 51.45% w/w and water soluble extractive value 49.75% w/w. The loss on drying value obtained is an indicative of amount of moisture content could prevent bacteria, fungal or yeast growth. Water soluble extractive value is higher than the alcohol soluble extractive value. The extractive values, indicates the amount of active constituents in given amount of plant material when extracted with respective solvent and useful for the determination of exhausted or adulterated drug. Ash values of the drug gave an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. total ash value is an indicative of total amount of inorganic material after complete incineration and the acid insoluble ash value is an indicative of silicate impurities, which might have arisen due to improper washing of the ingredients. Ash value is useful in determining authenticity and purity of the drug and also these values are important quantitative standards.

### Nutritional value analysis

Nutritional value analysis have been performed two samples of Anaemia control formulations (ACF1 & ACF2) separately in various parameters such as such as total fat % by weight, Protein ( $N \times 5.95$ ) % by weight, Total dietary fibre % Carbohydrates by difference, % by weight, Energy value K cal/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 1).

**Table 1 Nutritional value analysis of Anaemia control formulations (ACF1 & ACF2)**

S. No	Parameters	Unit of measurement	Results of ACF1	Result of ACF2
1	Total calories	Kcal/100g	395.10	459.66
2	Total carbohydrate	g/100g	45.60	57.18
3	Total fat	g/100g	17.40	19.94
4	Protein	g/100g	10.75	12.87
5	Vitamin C	mg/100g	2.00	2.13
6	Dietary fiber	g/100g	3.90	4.89
7	Iron (as Fe)	mg/100g	8.60	10.43
8	Calcium ( as Ca)	mg/100g	12.50	14.00
9	Vitamin A	IU/100gm	BLQ (10.0)	BLQ (10.0)
10	Sodium (as Na)	mg/100g	62.34	72.50
11	Potassium (as K)	mg/100g	105.60	140.80

**Preliminary phyto-chemical investigation**

Preliminary phyto-chemical analysis was performed in water and ethanol extracts. The result indicated that the alkaloids, carbohydrates, protein, resin, saponin, flavonoids, glycoside and resin are present in the aqueous extract and protein, alkaloids, resin, flavonoids, glycoside and resin present in the alcoholic extract of Anaemia control formulations (ACF1), while alkaloids, carbohydrates, protein, resin, saponin, flavonoids, glycoside and resin are present in the aqueous extract and protein, alkaloids, resin, flavonoids, glycoside and resin present in the alcoholic extract of Anaemia control formulations (ACF2) which could make the formulation potential and preventive health care.

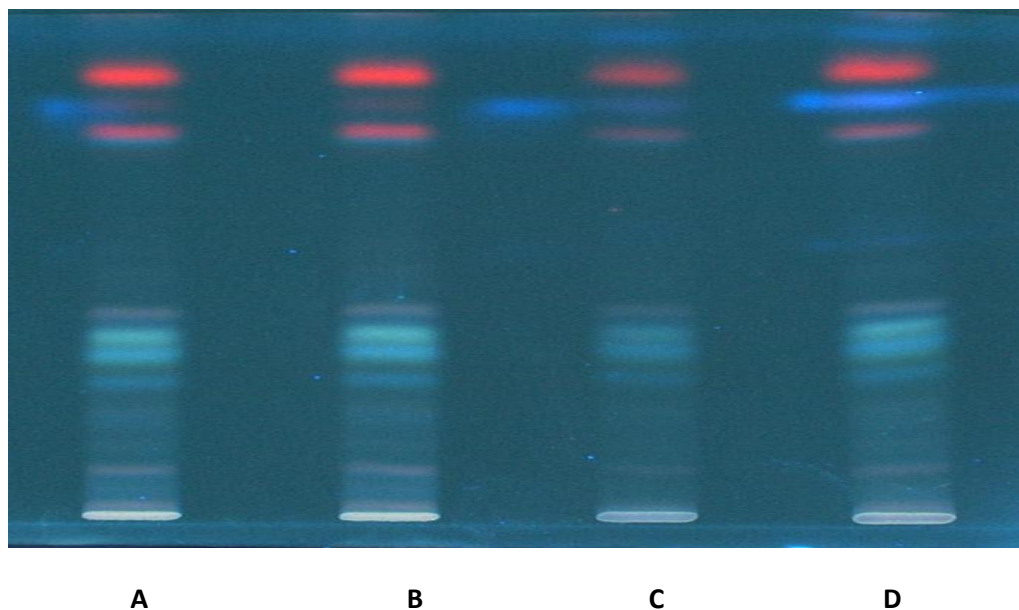
**Heavy metals tests**

Heavy metals (Pb, Cd, As and Hg) tests were performed in Anaemia control formulations (ACF1 & ACF2) and results were found under limits as per prescribed in WHO guidelines.

**HPTLC (high performance thin layer chromatography) finger prints profile**

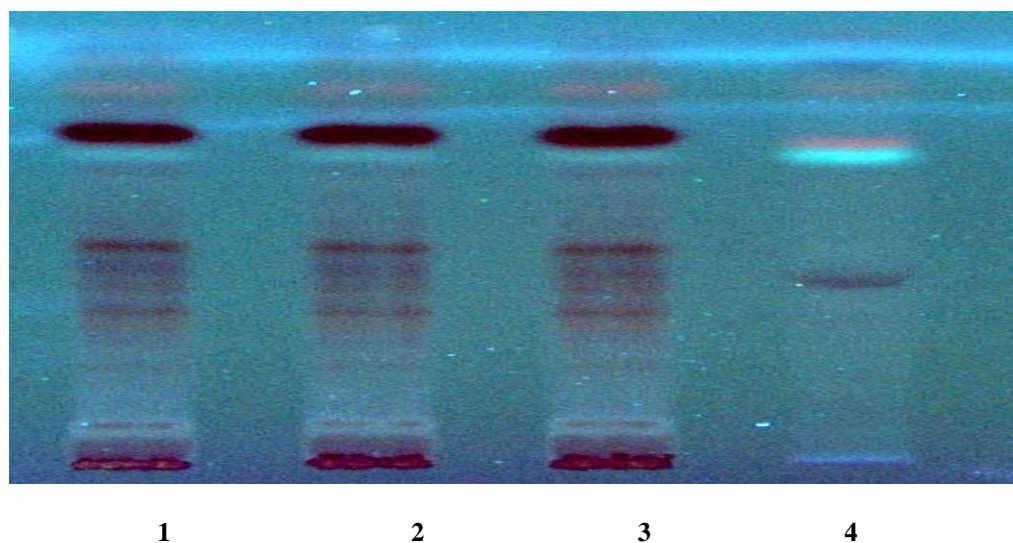
High performance thin layer chromatography (HPTLC) study of the methanolic extracts one spot of the formulation sample extract with ingredients spots applied in pre-coated TLC plate. Applied 6 µl of the test solution as 6 mm bands and developed the plate in a solvent system *toluene: ethyl acetate* (7: 3 v/v) to a distance of 8 cm. dried the developed plate in room temperature and examined. Derivatized the plate using 5% *Methanolic-sulphuric acid* reagent and dry at 105<sup>0</sup>C in hot air oven, till the bands were clearly visible. Major spots R<sub>f</sub> values with

colour were recorded before derivatization at 366nm and after derivatization at 366nm. Chromatograms profile and Rf values with colour are given (Fig.1 &2, table 2).



**Fig.1: HPTLC fingerprints profile of Anaemia control formulation (ACF1) at 366nm before derivatization**

**Abbreviations-** track- A formulation, track -B Satwar, track -C Ashwagandha, & track D Safed Musali,



**Abbreviation:** Track1-Compound formulation; track 2-test solution of Mahua; track-3-test solution of Til; track-4- test solution of Babool exude

**Fig.2: HPTLC fingerprints profile of Anaemia control formulation (ACF2) at 366nm after derivatization**

**Table-2: Rf values of HPTLC fingerprints profile of Anaemia control formulations (ACFI & ACF2 )**

Rf value	Anaemia control formulation (ACFI)	Anaemia control formulation (ACF2)
	At 366 nm, before derivatization	At 366 nm, after derivatization
Rf1	0.08 sky blue	0.10 brick red
Rf2	0.10 sky blue	0.12 brick red
Rf3	0.35 sky blue	0.35 brick red
Rf4	0.40 sky blue	0.40 brick red
Rf5	0.42 light brown	0.55 brick red
Rf6	0.75 red	0.80 brick red
Rf7	0.80 pink	0.86 brownish red

As per pharmacognostic study of the Anaemia control formulations (ACFI & ACF2 ) were standardized as per Ayurvedic Pharmacopoeia of Indian guidelines. The standardization were subjected to various analytical techniques like Organoleptic tests, microscopic tests, physicochemical tests, heavy metals tests, microbiological tests, nutritional value tests and pesticides tests. Powder microscopic test were performed and established the distinguished anatomical characters for two different samples (ACFI & ACF2) of Anaemia control formulations, results are depicted as (plate1&2), and these specific anatomical characters may be helpful for identification of ingredients in the Anaemia control formulations. Qualitative phyto-chemical analysis was performed in water and ethanol extracts. Various phytochemical like alkaloids, carbohydrates, flavonoids, protein, resin and saponin were present in both samples (ACFI & ACF2), which could make the formulation useful for potential and preventive healthcare needs. Detection of heavy metals (Pb, Cd, As & Hg) tests were performed and found below limits/absent as per WHO guidelines. Nutritional value analysis of two formulations of anaemia control samples (ACFI & ACF2) were done separately such total fat % by wt. 17.40%, 19.94%, Protein (N\*5.95) % by wt. 10.75%, 12.87%, Total dietary fibre % 3.90%,4.89 %, Carbohydrates by difference, % by wt. 45.60%, 57.18%, Energy value K cal/100mg 395.10, 459.66, Sodium mg/100gm 62.34, 72.50, Calcium mg/100gm 12.50, 14.00, Iron mg/100g 8.60, 10.43, Potassium mg/100gm 105.60 and 140.80 were found respectively. These findings indicate that formulations are potential used by tribal of Chitrakoot region to control the anaemia. These practices if integrated with modern healthcare system could elevate the health status of rural as well as urban children. This study may also helpful in the preparation of anaemia control formulations and their quality evaluation.

## CONCLUSION

From the present investigation documentation of folklore practices and pharmacognostic study of anaemia control formulations, various parameters such as documentation of traditional knowledge, selection of formulations, and its importance as a anaemia control used by tribal of

Chitrakoot region. Macroscopy, powder microscopic study, nutritional value analysis, physicochemical analysis, phytochemical investigation, heavy metal testing, and development of HPTLC fingerprints profile were carried out as per Ayurvedic Pharmacopoeial standards. The results of present study showed satisfactory as the efficacy and potency of the products as to control anaemia and immunity against common ailments of the human body. The developed folklore recipes can afford the health care needs in the society. The research outcomes of the documentation and standardization parameters can be used for evaluation the quality and purity of the anaemia control formulations (ACF1 & ACF2).

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### Conflict of interest

Authors declare no conflict of interest.

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