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Comparison and Efficacy Study of In-house Developed Formulation of *Fumaria indica* (Pitpapra) Against Expensive Market Alternative

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ABSTRACT

Extracts of Fumaria species have been traditionally used since ages in India, as they have potential for treatment of skin, scalp diseases, rheumatism, stomach ache and fever mainly because of the presence of many secondary metabolites which makes them pharmacologically valuable, specifically, alkaloids which are considered to have anti-inflammatory and analgesic properties. The purpose of this work was to compare *Fumaria indica* methanolic formulation with fumaria officinalis methanolic formulation using phytochemical profiling by HPTLC. Which showed that both the species are closely identical to each other. Therefore, this article suggests that *Fumaria indica* methanolic formulation can be used as an alternative of fumaria officinalis methanolic formulation as both have similarity in phytochemical ingredients when compared. In addition, the In-house developed formulation is economical and efficacious alternative towards market formulation.

Keywords: Herbal, Formulation, Anti-inflammatory, *Fumaria indica*, HPTLC.

INTRODUCTION

Fumaria indica (Hausskn.) Pugsley (Fumitory) (Synonyms: Fumaria parviflora var. indica, Basionym: Fumaria vaillantii var. indica) belong to family Papavaraceae, commonly known as Pitpapra in India according to Ayurvedic Formulary and the Ayurvedic Pharmacopoeia of India [1]. It is common annual herb weed found all over the plains of central Asia. As per, Ministry of Environment Forest & Climate Change, Botanical Survey of India, it is distributed in most part of Indian states including Bihar, Chandigarh, Delhi, Himachal Pradesh, Haryana, J & K, Karnataka, Maharashtra, Rajasthan, Punjab, Tamil Nadu, Uttarakhand, West Bengal. The genus is native to Europe, Africa and Asia and consists of around 60 species in world and commonly all the species of fumaria are known by the name of Fumitory[2]. The *Fumaria indica* is one of the most commonly used herbs and is reputed for its therapeutic properties include hepatoprotective, spasmogenic, anti-fungal, anti-bacterial and anti-inflammatory quality in the Indian traditional medicine structure[3,4]. Its medicinal values are often mentioned in ancient writing includes Charak Samhita, Bhava, Prakash Samhita and Dhanvantari Nighantu. In literature it is documented that *Fumaria indica* contains several alkaloids mainly isoquinoline alkaloids which are similar to Fumaria officinalis and Fumaria parviflora. Alkaloids mainly availed from plants are major class of molecules with anti-inflammatory activity, demonstrating inhibition of expression of several pro-inflammatory factors[5]. Fumaria species is known to calms irritation and itching induced by chemical hair treatment. Compounds such as phenols and polyphenols, carotenoids, proteins, peptides and polysaccharides have well-characterized anti-inflammatory activity.

Fumaria officinalis is widely available from Europe to Eastern Mediterranean. Also found at high altitudes in Nilgiris and Salem in Tamil Nadu, India [6]. It contains polyphenolic compounds like caffeic acid, rosmarinic acid, and apigenin, as well as alkaloids like fumaritrin, fumarofine, scoulerine, coptisine, protopine, fumaricine, fumaranine, fumariline and fumaric acid [7,8]. This plant is used for curing many inflammatory disease [9]. Mainly because of isoquinoline alkaloids and more specifically, exhibits anti-inflammatory activity, as observed in pharmacology research done in recent years [10]. An isoquinoline alkaloid, is one of the most medicinally dynamic phytochemicals, as it is ascribed to several pharmacological actions like antibacterial and antioxidant qualities [11] and are effective in treatment of eczema and other dermatological issues. As quoted in literature if its extract applied externally, it soothes psoriasis, eczema, scabies and other skin flare-ups due to presence of fumaric acid esters (FAEs).

HPTLC is one of the best techniques widely used in herbal and pharmaceutical industries for process development, identification, and detection of adulteration in commercial products and formulations. Thus, its separation and quantification can provide results that are either superior or comparable with other analytical methods such as HPLC. HPTLC is an extensively exploited due to fast, flexible, reliable,

cost-effective being with multiple sampling features. Here, chemical patterns are compared in correspondence with the standard to identify and estimate the markers in herbals. Now, it has become a precise analytical tool in routine analysis of botanicals, raw drugs, and finished formulation at micro or nano levels [12]. The HPTLC profile proposed for various plant extract are useful means for quality control and affirming batch to batch consistency of the plant material used by the herbal industries for manufacturing of herbal pharmaceutical products. In this article, we have compared the formulation claim to be made of Fumaria officialise (market product (MF)) with In-house made *Fumaria indica* formulation (RD05) by HPTLC fingerprinting method for phytochemical profiling and for knowing its pharmacology feasibility via literature data available. The focus of this study was to find cost effective alternative of market formulation without compromising the benefits and safety measure claim by the market available product.

MATERIAL AND METHOD

Chemicals: All the instruments and glassware (make- borosil) used during study were well calibrated. The solvent used were of analytical grade and purchased from Merck Ltd, Mumbai, India.

Preparation of plant extract: The fresh plant sample of *Fumaria indica* was collected from local market of Haridwar. The plant materials were cleaned, shade dried and coarsely grounded. Then, stored at dried and cool place until further use.

Extraction method

All the four trial of herb extraction using different menstruum mixture ratio were carried out in, in-house laboratory. Twenty gram each of herb powdered material were extracted using four different ratio of menstruum mixture (methanol: water). The menstruum mix used for all the four-extraction trial i.e. *Fumaria indica* extraction (FI-E) are Trial 1 (FI-ET1), Trial 2 (FI-ET2), Trial 3 (FI-ET3) and Trial 4 (FI-ET4) were in ratio 50:50, 60:40, 80:20 and 100% methanol respectively. For extraction the measured dried sample were loaded on reflux using 250 ml round bottom flask with 100ml of menstruum for 1.5hr at 45±2°C. The procedure was repeated thrice for all four-extraction trial. Further, each were filtered using whatman filter paper and combined. Then, vacuum dried on a rotary evaporator in order to obtain the dark brown colour dried powder. The powder obtained from each extraction trial FI-ET1, FI-ET2, FI-ET3 and FI-ET4 were FIF01, FIF02, FIF03 and FIF04 respectively were subjected to qualitative phytochemical study to know the presence of maximum secondary metabolites. Thereafter, among all recovered dried powder, the powder which has maximum clear bands on TLC and high in secondary metabolites content were mixed with vehicle solvent i.e., butylene glycol and water mixture (25:75), in relevant quantity. Further, the suitable preservative were added in limit prescribed as per authorized official guidelines. The HPTLC fingerprinting investigation of formulations were performed to compare RD05 with market purchased formulation used as one of the ingredients for hair colour cream. Moreover, the pharmacological authenticity of RD05 were confirm by literature data available.

Preliminary Phytochemical screening

Qualitative phytochemical analysis was performed to determine the presence or absence of secondary plant metabolites in *Fumaria indica* methanolic extract using standard methods [13,14]. Secondary metabolites are chemical compounds derive from subsidiary pathway as shikimate pathway. They have shown to process various biological effects, which provide the scientific base for medical efficiency of herb.

Chromatographic conditions

HPTLC densitometry analysis was preform on precoated silica gel aluminium plate 60F-254 (10cm x 10 cm with 0.2mm thickness, E. Merck, Germany) using CAMAG Linomat-V. One gram of each dry powder (FIF01, FIF02, FIF03 and FIF04) and one gram of prepared RD05 were dissolve in 10ml alcohol, separately and sonicated, then were employed as bands of width 6 mm with a CAMAG microliter syringe with a constant application rate of 150 nL/s. The linear ascending development was carried out in trough optimized chamber saturated for 10 minutes with the mobile phase consisted of Toluene: ethyl acetate: formic acid (5:4:1 v/v/v, 20 mL). The mobile phase selected among different mobile phase tested during study on basis of maximum and clear band separation. Thereafter, TLC plates were dried in oven at 105°C for two minutes. Densitometry scanning was performed on CAMAG TLC scanner III in the absorbance mode at 254 and 366 nm.

RESULT AND DISCUSSION

The extracts of *Fumaria indica* so obtained after each extraction process were weighed and their yield percentage were recorded. The percentage yield of extraction is very important in phytochemical extraction in order to evaluate the standard extraction efficiency for any herbal extraction. The yield of extracts obtained from all the four-extraction trial were depicted in Table 1. Among four extraction performed refluxing with 100% methanol (FI-ET4) provided highest extracted yield of *Fumaria indica*. Further, the preliminary phytochemical and HPTLC analysis of all the dry powder FIF01, FIF02, FIF03 and FIF04 obtained were performed. The presence of separated bands for selecting the best mobile phase system in HPTLC all the four powder samples were tested for different mobile phases. Based on available literature reviews, various combinations of mobile phase systems were studied with an aim to have an appropriate mobile phase composition for the best and most efficient HPTLC chromatographic separation of efficient resolved bands. Among all the mobile phase combinations studied, Toluene: ethyl acetate: formic acid (5:4:1 v/v/v) was finalized to be the ideal solvent system for the evaluation of maximum and clear resolved bands. Among all four samples the FIF04 confirm the presence of maximum number of secondary metabolites which include alkaloids, carbohydrates, flavonoids, glycosides, polyphenols, quinones, tannins and triterpenes and therefore depicted maximum and clearly separated bands on TLC.

Table 1: Result of percentage yield of plant extraction

Trial	*Fumaria indica* extract		% Yield
	Solvent	Solvent ratio	
T1	FI-ET1	50:50	8.50
T2	FI-ET2	60:40	10.10
T3	FI-ET3	80:20	11.64
T4	FI-ET4	100	14.40

Therefore, FIF04 were selected for formulation development and was further used for comparison with market bought formulation. Now, work was to establish a HPTLC method for fast screening and comparing the similar bands between the two formulations, RD05 and market formulation. Depending on number of similar spots, we can examine the similarity between the samples compared. Most of the spots has same retention factor (Rf) in both samples, whereas fewer has different Rf value. The HPTLC chromatogram clearly depict in Figures (1 and 2) that there is a close affinity between the two samples (Market formulation (MF) and In- House formulation (RD05) when compared.

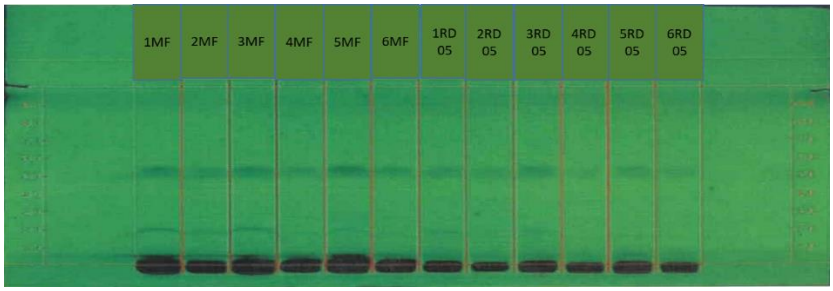

Figure 1: Comparison of both formulation at 254 nm

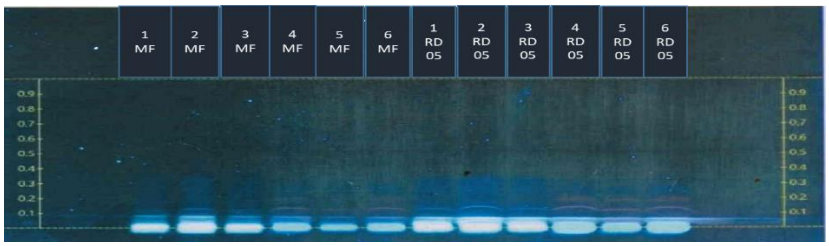

Figure 2: Comparison of both formulation at 366 nm

Hence, both the formulations can replace each other as potential substitute. The fumaria herbs from long ago were used for the treatment of inflammatory conditions as household remedies. It reduces the inflammatory reaction by limiting the release of key inflammation factors such as NF-kB (Nuclear factor kappa B) and PGE_2 (Prostaglandin E2)[9]. It was, therefore, considered for use in cosmetics and hair products in worldwide laboratories. Several pharmacological properties of *Fumaria indica* extracts were similar to those reported extracts from other members of fumaria species having phytochemical constituents like alkaloids, tannins, triterpenoids, steroids which has huge medicinal value [16]. Further, Fumaria species pharmacological efficacy were screened through data provided by many researchers based on clinical trials. The study conducted by C.V Rao *et. al.* [14], examine hydro- alcoholic extract of *Fumaria indica* for its anti-inflammatory affinity in animal models. The administration of dried extract through three doses 100, 200 and 400 mg kg^{-1} orally demonstrated doses dependent and significant anti-inflammatory activity in acute (carrageenean and histamine induced hind paw oedema, $p < 0.05$) and chronic cotton pellet granuloma models of inflammation, ($p < 0.01$). All the doses tested has more or less anti inflammation activity but dose 400 mg kg^{-1} exhibited maximum anti-inflammatory effects after 3hr with carrageenean and histamine, respectively. The W. Rizvi *et. al.* [15], experimented on leaves extract of Fumaria parviflora for anti-inflammatory activity. The test method demonstrated significant ($p<0.001$) reduction in paw edema in carrageenan-induced paw edema. In addition, it also represents significant decrease in granuloma formation in cotton pellet-induced granuloma method. The research done by A. Shakya *et. al.* [10] confirm that *Fumaria indica* (FI) and Fumaric acid (FA) show anti-inflammatory activity, even after their minimum doses were applied. Which were demonstrated using carrageenan- induced edema and cotton pallet granuloma test model. The phytochemicals present, the comparison using HPTLC suggest that *Fumaria indica* structurally similar to those of many other plants of fumaria species, additionally their pharmacological data demonstrate their efficient affinity as anti-inflammatory.

CONCLUSION

The practice of using medicated herbs has been in existence since long ago. In today's time there has been a steady rise in the dependence on the use of plants and herbs in industries and research laboratories as they are rich source of potential phytochemicals. Therefore, they are best substitute available as naturally occurring alternative against synthetic product. The fumaria species known to have rich source of alkaloids, which are considered to be anti-inflammatory, by many researchers globally. Pharmacological investigation of anti-inflammatory properties through animal model of Fumaria species has been confirmed by literature data available [5]. The phytochemicals profile of *Fumaria indica* are closely identical to those of many other plants of fumaria officialise. The current investigation indicated the similarities between the In-house developed and market available formulation using HPTLC and pharmacological study. It can be concluded from this study that In-house formulation can be an alternative for market available formulation. Moreover, In-house formulation may be an efficient and cost-effective option in place of expensive market formulation. Thus, further study is needed towards the more data generation for potential evaluation of fumaria species, considering its anti-inflammatory and other beneficial properties, identification of bioactive ingredients and explication of their mechanism of action for developing more eco-friendly and healthy herbal products.

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Conflict of interest

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