

Formation of an Ointment from the Herbal Sources of Nikadi Pottani and Identification the Contribution of Each Source toward the Active Ingredients of the Final Product with TLC Finger Prints

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Abstract: There is a tendency of increasing of neurological and inflammatory disorders in society. Thus, the study was carried to prepare a user friendly Analgesic, Anti-inflammatory ointment from the traditional Ayurvedic Thavili Pottani used for neurological and inflammatory disorders. Contribution of active ingredients from each plant source was also tested with TLC fingerprints. Study was carried in Dravyaguna laboratory (April, 2019 to April, 2020). Dried plant parts of *Vitex nigundo*, *Adhatoda vasica*, *Ricinus communis*, *Alpinia galanga*, *Brassica nigra*, *Allium sativum* were fine powdered and mixed up. The mixture was soxhlet extracted with ethanol. The extract was mixed-up with ointment base containing bee's wax, liquid paraffin, methyl paraben and propyl paraben and prepared the ointment. Secondary, the powdered mixture as well as each powdered plant material was soxhlet extracted ethanol separately and concentrated with rotary evaporator. Eventually a 10 µl of each was placed in a same TLC plate and run in a chamber (mobile phase = Toluene: Ethyl acetate = 13:1). Under TLC fingerprints, visible bands were found for the mixture (six) as well as each plant source. Out of the six bands of mixture, two were tallied with the two bands of *Vitex*, and *Alpinia*. Three bands were tallied with three bands of *Adhatoda*, and *Ricinus*. One band was tallied with a band of *Brassica*. Hence, among the ingredients of final product, highest contribution was from *Adhatoda*, and *Ricinus*. Second most was from *Vitex*, and *Alpinia* and third most contribution was from *Brassica*. The contribution from *Allium* was invisible and this could be due to volatile nature of the ingredients.

Keyword: *Nikadi Pottani, Dry powder, Soxhlet extract, Ointment, Anti-inflammation.*

Article Received: 05 Dec. 2020

Revised: 15 Dec. 2020

Accepted: 25 Dec. 2020

Introduction

Inflammatory and neurological disorders are commonly seen in the modern society. Ayurveda is a holistic system which describes strongly the promoting, preventive and curative aspects of the personal health. Pottani preparations, which come under *Swedana* and *Sneha* treatment regime, are used basically in Ayurveda for treating neurological disorders.

Nikadi Pottani is also one such and used in Ayurveda to heal neurological and inflammatory situations. In the preparation of Nikadi Pottani, fresh herbs (Table 1) are used in appropriate quantities as mentioned in the recipe [1]. Then these herbs are crushed and made Pottani and continued the procedure called Wandu Thembum.

Table 1: Components of Nikadi Pottani

Scientific name of the plant source	General name	Plant part used in
<i>Vitex nigundo</i>	NIKA	Leaves
<i>Adhatoda vasica</i>	ADATHODA	Leaves
<i>Ricinus communis</i>	ERADU	Leaves

Alpinia galanga	ARATTA	Rhizomes
Brassica nigra	ABA	Seeds
Allium sativum	SUDULUNU	Bulbs

Though this preparation has been used from ancient time, in the modern society it is somewhat cumbersome to find fresh real quality herbs and then prepare the Pottani. Further, sometime the same Pottani has to be used repeatedly for several days, which in turn to less effective the treatments. Moreover, it is poorer in sanitary standard as well. Thus, the concept of preparation of user friendly anti-inflammatory ointment with the same ingredients of Nikady Pottani was minded and this in vitro research was carried out as result of that to make the concept success.

When comparing Nikadi Pottani and ointment, both advantages and disadvantages are found. By using as an ointment, the homogeneity of drugs used here are preserved and also by using as fresh ingredients, it will increase the efficacy of the drug. When the fine powders of ingredients are mixed well with ointment base (lipid), the lipid soluble active are dissolved well in the ointment. In the Pottani, as aqueous medium is used, only the water soluble components come in to react.

Anyway, as the Pottani is used in hot condition, the absorption of ingredient to skin is enhanced due to the improvement of blood supply to the applying area. This is an advantage of the Pottani method. Anyway, this can be overcome in the ointment procedure, by applying the ointment and rubbing the area well. The addition of perseverative can minimize the microbial attack on active ingredient of the ointment, this is not met in the traditional Nikady Pottany method.

Furthermore, the volatile component of the plant sources can be lost in Pottany method which is minimized in the ointment method. Anyway, for the development of the traditional medical system, the discoveries are must. In addition to the ointment preparation, to find out the contribution of active ingredients from each plant source, the TLC fingerprints were carried out. Thus, the TLC fingerprints were carried out for each mixture as well as the plant sources separately to compare the bands, hence to achieve the objectives.

Method

Preparation of Herbal Extract [2]

All the six plant materials such as leaves of *Vitex nigundo*, *Adhatoda vasica*, *Ricinus communis*, Rhizomes of *Alpinia galanga*, bulb of *Allium sativum*, and seeds of *Brassica nigra*, were cleaned and removed the external substances. Then they were dried under shade. The dried plant materials were separately milled and made into coarse powders until to be filtered through sieve no.20 sieve.

Thereafter 50 g of each ingredient was mixed to form the final drug mixture. Exact 250 g from final mixture was extracted with hydro alcoholic mixture (1000 ml, 90:10 v/v Ethanol: water) at 70°C for 24 hrs using the soxhlet apparatus and the resultant extract was 200 ml. This was further concentrated by rotary evaporator at 40°C for 6 hrs till the final extract was 50 ml.

Formulation of Ointment Base and Preparation of Herbal Ointment

Bee wax and liquid paraffin were heated separately in china dish at 70°C. Methyl paraben and propyl paraben were mixed separately. Bee wax and liquid paraffin were added drop by drop in to the methyl paraben mixture with constant stirring to form an ointment base. Final ointment base was 25.2 g. Ointment base (25 g) was mixed up with 0.3 g of Ethanolic extract while stirring to make 25.3 g of final ointment.

TLC Finger Print Profile [3]

Coarse dry powder of each six plant material (25 g from each) was extracted separately with hydro alcoholic mixture (100 ml, 90:10 v/v Ethanol: water) at 60-70°C for 24 hrs by continual hot extraction method using soxhlet apparatus and concentrated by rotary evaporator as above.

A drop of each individual extract (10 µl) and extracted final drug preparation were applied separately on TLC plates with a capillary tube and the plates were developed up to a distance of 10 cm at 25±2°C in a chamber containing mobile phase (Toluene: Ethyl acetate 13:1) ⁽²³⁾ and 40% relative humidity.

Aqueous extract of final drug mixture was also run in the same TLC plate.

Plates were dried at room temperature and observed for band. The Rf values of bands were noted.

Result



Figure 1: The final product

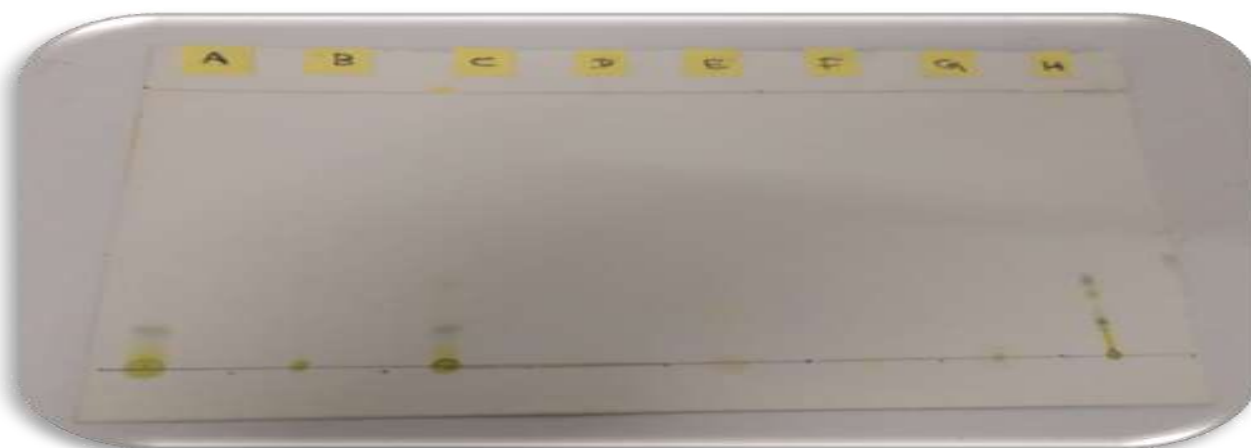


Figure 2: TLC finger print for the whole mixture and each component separately

A: *Adathoda* Leaves extract

B: *Vitex* leaves extract

C: *Ricinus* Leaves extract

D: *Allium* Bulb extract

E: *Alpinia* Rhizomes extract

F: *Brassica* Seeds extract

G: Aqueous extract of final mixture

H: Ethanoic extract of final mixture

Discussion

The final ointment (Figure 1) was prepared successfully according to the reference method and was semi solid and was easy to be applied. According to the TLC fingerprint (Figure 2), six bands were visible in the final drug powder mixture. Thus, the nonvolatile ingredients noticed under the study was six. Out of them, two bands ($R_f = 0.27, 0.12$) were tallied with the two bands of each *Vitex nigundo*, and *Alpinia galanga*.

Thus, the contribution of ingredients to the final mixture from *Vitex nigundo*, and *Alpinia galanga* was similar and two from each. Three bands of the final mixture ($R_f = 0.23, 0.11, 0.06$) were tallied with the three bands of *Adhatoda vasica*, and *Ricinus communis*. Hence, the highest contribution of ingredients to the final product was seen

from *Adhatoda vasica*, and *Ricinus communis* and was three similar ingredients from each. The least contribution was seen from *Brassica nigra* and was only one ingredient (one band of final mixture was tallied with a one band of *Brassica nigra* mixture, $R_f = 0.07$). The contribution from *Allium sativum* was invisible and could be due to volatile nature of the ingredients.

Further, it was noticed that the TLC fingerprint of aqueous extract of final mixture had only few bands. Hence most of the ingredients of final product is soluble in polar solutions. According to the scientific studies, it has been found that the plant components of the *Nikadi Pottani* contains anti-inflammatory [4] anti-allergic, anti-analgesic and anti-arthritic actions [5,6].

It has been found to have allicin (diallyl disulfide) in the *Allium sativum*, which is volatile in nature, causes anti inflammation actions via inhibiting the pro-inflammatory cytokines (NFκB, iNOS and TNFα) [7]. This volatile nature could be the reason for not appearing a band in the TLC fingerprint for *Allium*. Thus, it is a must to preserve the allicin in the ointment by adding *Allium* itself without doing extraction processes.

The studies have showed that the flavonoids [8] found in *Brassica nigra* has anti-inflammatory effect to reduce inflammation. The chemicals such as 1'S-1'-acetoxychavicol acetate, 1'S-1'-acetoxyeugenol acetate, and p-hydroxy cinnamaldehyde, found in *Alpinia galanga* have expressed major anti-inflammatory and anti-allergic qualities (blocking histamine, serotonin and beta-hexosaminidase) [9] *in vivo* and *in vitro*. Chemical compounds naming lupeol and diketone pentacyclic triterpene (erandone) found in the *Ricinus communis* reduce the inflammation [10].

Adathoda vasica has also exhibited remarkable anti-inflammatory activities via an alkaloid naming vasicine [11]. Polyphenols (as gallic acid equivalents) found in the *Vitex nigundo* has showed potent antiallergic, anti-inflammatory actions via inhibiting prostaglandin synthesis [12].

Conclusion

The ointment, which was developed, is a novel product. As the TLC studies have shown an active ingredients of the final product and as the certain previous studies have proved that each plant components of the final product contains ingredients which can cause anti-inflammatory, analgesic, anti-allergic effect, the product could be basically effective as an external ointment for the soft tissue inflammation (anti-inflammatory agent). Furthermore, it is expected to cover an *in vitro* and *in vivo* study (toxic study, clinical studies etc.) for the ointment for a better efficacy and effectiveness.

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