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SHORT COMMUNICATION

INSIGHT INTO THE QUALITY OF NEELAMARI LEAF POWDER: A MARKET-BASED COMPARATIVE STUDY

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Abstract: Neel (*Indigofera tinctoria* L.), commonly known as true indigo and belonging to the Fabaceae family, is a historically known natural source of blue indigo dye. Beyond its dyeing properties, it also possesses notable medicinal value. The leaves of Neelare key components in several Ayurvedic formulations, like *Aravindasavam*, *Neelibringadi oil*, *Neelithulasyadi kashayam etc.* With the increasing commercial demand, numerous branded indigo leaf powders are available in the herbal market, but concerns over adulteration persist. This study, conducted at Kerala Agricultural University, Vellanikkara, Thrissur, aimed to evaluate the quality of marketed Indigo leaf powder samples using High-Performance Thin Layer Chromatography (HPTLC). Samples were collected from herbal outlets across Kerala as well as from online platforms, grouped to nine and compared against a reference standard. HPTLC analysis revealed that while majority of the samples were authentic, certain brands showed evidence of adulteration or mixing. The study underscores the effectiveness of HPTLC as a reliable tool for authenticating herbal powders and highlights the need for stricter quality control in the herbal raw drug market.

Keywords: *Indigofera tinctoria*, Neelamari, Indigo leaf powder, HPTLC, Quality evaluation, Adulteration

INTRODUCTION

I*Ndigofera tinctoria* L., a member of the Fabaceae family, is widely recognized for its role as a natural dye source and for its medicinal applications in traditional Indian medicine. This plant is called as Neel in Hindi, Neelini in Sanskrit and Neelamari in Malayalam. In India, Neel is cultivated in the states of Assam, Andhra Pradesh, West Bengal, Bihar, Tamil Nadu and Kerala. It is a branching shrub growing up to about 2 m height. In Ayurvedic system of medicine, Neel leaves are used as a major ingredient in *Neelibhringadi* oil, *Neelithulasiadi thailam, Neelithulasiadi kashayam, Aravindasavam* etc. The Juice of leaves mixed with honey is effectively used against enlargement of liver and spleen, for epilepsy and for nervous problems (Kashyap *et al*., 2006 and Tomar, 2006). The medicinal properties of *Indigofera tinctoria* are attributed to its rich chemical profile. The active compounds include indigotin, flavonoids, tannins, triterpenoids, saponins, glycosides and alkaloids. Indican ($C_{16}H_{11}O_7N_2$) is the glycoside precursor to indigotin (Mishra *et al*., 2020). With increased demand, numerous indigo leaf pow$_2$) isss brands have entered the hindigotin ((et, particularly for natural hair dye and for preparation of other formulations. However, adulteration remains a critical challenge affecting the efficacy and safety of these products. The present study utilizes High-Performance Thin Layer Chromatography (HPTLC) to assess the authenticity of commercial indigo powders, comparing them against authenticated reference material (Gunjal *et al*., 2022).

MATERIALS AND METHODS

Collection and preparation of samples

Authentic leaves of *Indigofera tinctoria* were collected from the experimental farm of the College of Agriculture, Kerala Agricultural University, Thrissur. The plant material was botanically authenticated, shade-dried, and powdered to serve as the reference sample. A total of 57 commercial indigo powder samples were procured from online platforms, herbal outlets, and local stores across

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Kerala. These were grouped under 9 different branded categories for analysis (Figure 1).

Extraction procedure

Five grams of each powdered sample was refluxed overnight with 50 mL of methanol. The extracts were filtered, concentrated under vacuum, and stored for doing HPTLC analysis.

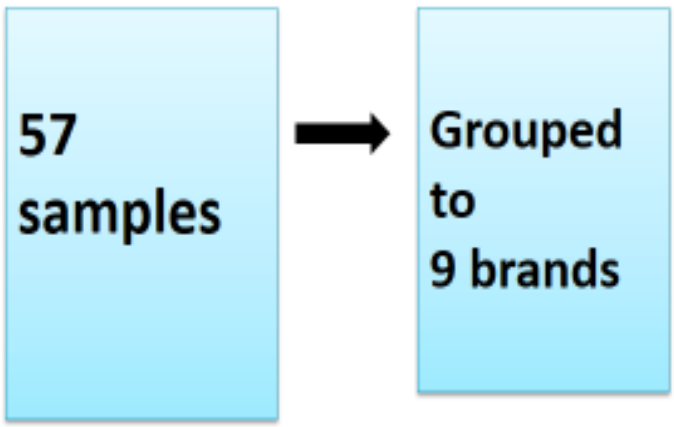
HPTLC analysis

HPTLC fingerprinting was conducted using pre-coated silica gel 60 F254 plates as the stationary phase. The mobile phase consisted of toluene: acetone: formic acid (4.5:4.5:1). Plates were developed in a CAMAG twin-trough chamber (10×10 cm) pre-saturated with the mobile phase for 15 minutes and developed up to 8 cm. Developed plates were dried and visualized under short-wave and long-wave UV light. Rf values and band profiles of the market samples were compared against the genuine reference to assess sample purity and detect potential adulteration or admixing (Gunjal *et al*., 2022; Harborne, 1998; Wagner and Bladt, 1966).

RESULTS AND DISCUSSION

The HPTLC profile of the authentic (Genuine material) *Indigofera tinctoria* leaf powder served as the reference standard. Comparative analysis of market samples revealed that 8 out of 9 branded powders matched the reference profile, while one sample (Brand IT3) displayed additional bands indicative of adulteration or admixture (Figure 2). Hence it can be assessed that among the total 57 samples analyzed, approximately 89% were confirmed as genuine, while 11% exhibited signs of mixing or substitution. Table .1 summarizes the findings and this study reflected the current scenario of the herbal raw drug trade of Neel in market, emphasizing the risk of adulteration even in well-labeled products. The results also highlighted the utility of HPTLC as a quick, reproducible, and reliable method for screening herbal raw materials. Ensuring authenticity through such analytical tools is vital for protecting consumer health and maintaining trust in traditional medicine.

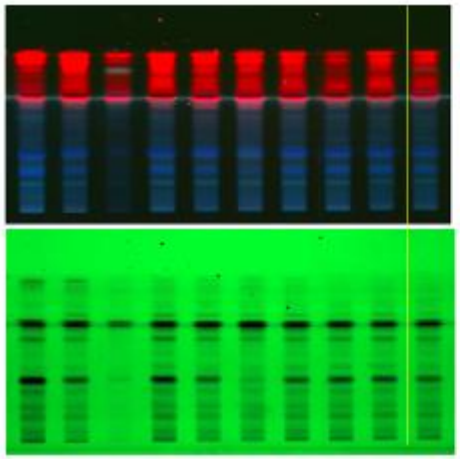
Fig 1.Market sample analysis-Neel powder



Group code No:
IT 1
IT 2
IT 3
IT 4
IT 5
IT 6
IT 7
IT 8
IT 9
IT 10 Reference sample –Genuine from KAU farm

17

Fig.2.Chromatogram of Neel leaf powder market samples in comparison with reference sample



Sample group code No:	Assessment on quality
IT 1	True sample
IT 2	True sample
IT 3	Impure sample
IT 4	True sample
IT 5	True sample
IT 6	True sample
IT 7	True sample
IT 8	True sample
IT 9	True sample
IT 10	Reference sample (True sample)

Methanol extract,Silica gel 60 F_{254}, Mobile phase (Toluene:acetone:Formic acid 4.5:4.5:1),UV-L ,UV-S

CONCLUSIONS

This study confirmed that while most commercial *Indigofera tinctoria* leaf powders in the herbal market are authentic, some instances of adulteration persist. HPTLC proved to be a powerful tool for evaluating the quality and authenticity of Neel leaf powders. These findings underscored the importance of implementing routine quality checks and establishing regulatory standards for herbal raw drugs. Mandatory quality assurance practices and accurate labeling will help preserve the integrity of traditional herbal systems and ensure consumer safety.

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