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Research Article

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### Comparative Phyto-Constituent study of Old and New *Pippali* Fruits by HPTLC Analysis

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#### Abstract

The overall world and whole universe is endowed with many therapeutic herbs. These therapeutic herbs aid in the treatment of various illnesses. *Pippali* (*P. longum*) is one such natural herbal plant; this plant is used as a primary ingredient in several formulations in ancient Ayurveda to treat digestive and respiratory issues as well as numerous *Kaphaja Vikar*. In Ayurveda, it is used as a *Rasayana* to cure a variety of illnesses. In many *Avaleha* and preparations, it is used as *Prakshepak dravya*. The pharmacological characteristics of *Pippali* include analgesic, anti-inflammatory, antibacterial, antidiabetic and hepato-protective effects. *Pippali* contains many chemical constituents which include flavonoids, piperine and terpenoids, etc. The standardization and quality analysis of such herb is prerequisite to ensure their compositions and properties. In this regard advanced analytical techniques like HPTLC can be utilized. Considering this fact present study compares the phytochemical components of fresh and old *Pippali* fruits, highlighting variations in bioactive compounds brought on by aging and their effects on inherent properties.

**Keywords:** Ayurveda, HPTLC, Standardization, Pippali, Phytochemical

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#### 1. Introduction

Ayurveda is one of the oldest Indian medical traditions, which explored several natural compounds for therapeutic purpose. *Pippali* is one such compound used for a variety of ailments including *Shwasa*, *Kasa*, *Gulma*, *Meha*, *Arsha*, *Pliha*, *Udar*, *Kushtha*, *Shula*, *Jwara*, *Aruchi*, *Krimi*, *Pandu* and *Kshaya*, etc. Its botanical name is *Piper longum*, and its roots and fruits are used for therapeutic purposes. It used with various forms of *Anupan* such as *Dugdha*, *Ghr̥ita*, *Madhu*, *Guda* and *Samanya jala*. It alleviates vitiated *Pitta*, *Vata* and *Kapha Doshas*, also used as a blood purifier and brain tonic. It is used as a moderate laxative and to relieve pain in the stomach. *Pippali* reduces hiccups and has anti-tussive and anti-asthmatic qualities. As a respiratory tonic it alleviates diseases of respiratory tract. (1-4)

The therapeutic action of *Pippali* mainly governed by its Ayurvedic properties (*Rasapanchak*) which includes *Laghu*, *Tikshna* and *Snigdha Guna*, *Katu Rasa*, *Madhur Vipak* and *Anushnasheet Veerya*. The efficacy of its

therapeutic action depends largely on its phyto-chemical constituents, which can degrade or change over time as a result of environmental conditions, storage, and oxidation. (3-5) Therefore, comparison of new and old *Pippali* is prerequisite to ensure their therapeutic efficacy.

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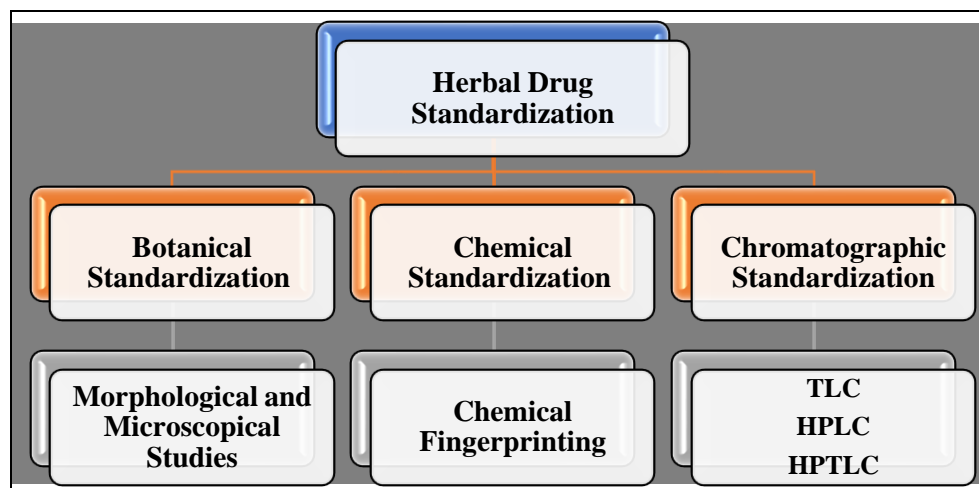
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This *Shloka* of *Sharangdhar Samhita* brings forth the usage of fresh medicines but clearly states certain exceptions such as *Vidanga*, *Pippali*, *Guda*, *Dhanya* and *Madhu* that are deemed more effective after aging [6]. The rule proves how crucial it is to realize the strength and lifespan of various components in Ayurvedic formulations.

Drug, standardization is a crucial component of polyherbal formulation with respect to their quality and safety concern. A system of standardization for all plant medicines currently on the market is crucial because there is a great deal of variation from batch to batch. Plant

material can result in a variant therapeutic effect based on variant collection batches, including collection at another season or from locations with another environmental

location. (7,8) Development of scientific field evolved several techniques for herbal drug standardization as mentioned in **Figure 1**.



**Figure 1.** Various approaches of herbal drug standardization

As mentioned above in Figure, High-performance thin layer chromatography (HPTLC) is one of the sophisticated instrumental methods for qualitative and quantitative determination of the herbs and herbal drugs. The physicochemical parameters are described in depth in certain pharmacopeias that include monographs on plant material. In order to preserve the consistent quality of herbal preparations, chromatographic fingerprinting has been offered as a method for identification and characterization. (9,10) HPTLC is a sophisticated TLC analysis that provides better resolution and can quickly and accurately estimate the active components. One significant benefit of HPTLC is its ability to analyze multiple samples at once with minimal mobile phase usage. This reduces the risk of causing environmental pollution, saves time and money on analysis, and reduces exposure hazards and toxic organic effluent disposal issues. (11)

The efficacy and safety of herbal drug like *Pippali* depends largely on its phyto-chemical constituents, which can degrade or change over time as a result of environmental conditions, storage, and oxidation. Considering this fact present study was planned which utilized chromatographic technique to examine the phyto-chemical differences between new and old *Pippali* samples.

## 2. Materials and Methods:

### Sample Collection and Preparation

- Sample Type: Coarse dried black powder of Pippali (New & Old)
- New – Fresh Pippali Fruits collected and dried
- Old – fruits of Pippali collected, dried and kept for one year
- Sample Weight: 200 mg each
- Solvent for Extraction: Water: Acetonitrile: Methanol (1:2:2 v/v), 5 ml
- Sample Concentration: 40 mg/ml (40,000 ppm)
- pH: 29°C.

### Chromatographic Analysis Parameters

- Instrument: Shimadzu 10Avp (Quaternary Pump, UV/DAD detector)
- Column: UltraSil RP (5 µm, 150 × 4.6 mm ID)
- Mobile Phase: 15mM KH<sub>2</sub>PO<sub>4</sub> - Methanol (30:70 %, v/v)
- Temperature: 28°C
- Wavelengths: 210 nm & 254 nm
- Flow Rate: 1.0 ml/min
- Injection Volume: 20 µl
- Run Time: 75 minutes

Sample collection and preparation included coarse dried black *Pippali* powder, both new and old, with each sample weighing 200 mg. The extraction solvent used was a 1:2:2 mixture of water, acetonitrile, and methanol in a ratio by volume, having a total of 5 ml. The resultant sample concentration was 40 mg/ml (40,000 ppm), and the process was performed at a temperature of 29°C.

For chromatographic separation, an instrument Shimadzu 10Avp fitted with a quaternary pump and UV/DAD detector was employed. Column separation was conducted using an UltraSil RP column (5 µm, 150 × 4.6 mm ID). The mobile phase was 15 mM KH<sub>2</sub>PO<sub>4</sub> and methanol in the ratio of 30:70 (v/v). Column temperature was kept at 28°C, and detection was monitored at wavelengths of 210 nm and 254 nm. Flow rate was established at 1.0 ml/min, injection volume was 20 µl with run time of 75 minutes.

## 3. Results and Discussion

The study revealed the presence of various bioactive compounds, with notable differences between old and new *Pippali* samples as mentioned in **Table 1**.

Analysis of piperine content showed that new *Pippali* fruits had 0.67% piperine (0.67 mg/100 mg sample) whereas old *Pippali* fruit sample had slightly reduced piperine content as 0.60% (0.60 mg/100 mg sample) which showed the loss of some amount of piperine content during aging. Both samples had a single phenolic

acid present, but their identification could not be done. Flavonoids were there but were not the usual flavonoids like rutin, myricetin, galangin, and chrysin. They also found a varied mix of 21–23 terpenoids making up around 50% of the content in both samples. Steroids were not found in either sample.

**Table 1.** Phytochemical observation in *Pippali* samples

S. No.	Phytochemical	New Pippali (%)	Old Pippali (%)
1	Piperine	0.67	0.60
2	Terpenoids	~50%	~50%
3	Flavonoids	Present	Present
4	Phenolic Acids	Present	Present
5	Steroids	Not detected	Not detected

#### 4. Discussion

Study observed slightly greater concentrations of piperine and other bioactive ingredients exist in Fresh *Pippali* when compared with older *Pippali* sample. Terpenoids, accounting for approximately 50% of the overall composition, are stable in both samples. Flavonoid composition was found in both but with differences in constituents and no steroidal compound was found in either sample.

This fresh *Pippali* has *Ushna Virya*, *Madhura Vipaka*, *Katu* and *Madhura Rasa*. It offers potent *Vata-Kapha Shamaka* and *Deepan-Pachan* effects by virtue of these properties. With time, as the *Pippali* grows old, its nature evolves and pungency becomes intense, and it acquires a *Katu Vipaka*, becoming keener in activity with an intensified *Lekhana* property. This modification strengthens its activity in *Kapha* disorders and renders it more efficient as a *Medohara* agent.

From the perspective of the modern drugstore, fresh *Pippali* has a higher volatile oil content, making it more fragrant and powerful in stimulating digestive and respiratory functions, and a more intense alkaloid content, consisting of piperine and pellitorine, which is responsible for its bioavailability-enhancing, expectorant, and bronchodilator activities. However, as *Pippali* ages, its volatile oil concentration falls, which makes it less effective for digestion but more effective in reducing fat deposits and excess *Kapha*.

These factors make fresh *Pippali* useful for respiratory conditions, rejuvenation therapy and weak digestion, while old *Pippali* is utilized for metabolic disorders and obesity, etc. Thus, the choice between using fresh or old *Pippali* fruits depends on the need of specific therapeutic goal. New *Pippali* fruit advises for digestive issue while old *Pippali* fruit is considered good for disorders of *Kapha* and *Meda Dhatu*.

#### 5. Conclusion

When fresh and aged *Pippali* fruits are compared, the terpenoid content remains unchanged, but the piperine content decreases with age. The beneficial components in freshly harvested *Pippali* are slightly more abundant, suggesting that storage duration may influence its potency. This study emphasizes the necessity of ideal

storage conditions to guarantee phytochemical stability and supports the Ayurvedic practice of using fresh herbs for the best therapeutic activity. The *Sharangdhar Samhita* also emphasizes uses of fresh medicines except for *Vidanga*, *Pippali*, *Guda*, *Dhanya* and *Madhu*.

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#### Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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