

## PHARMACOGNOSTIC EVALUATION OF PIPPALI MULA (ROOT OF *Piper longum* Linn.) W.S.R. TO MICROMETRIC AND ISOLATION TECHNIQUES

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### Abstract

Pippalimula (root of *Piper longum* Linn.) is one of the vital herbs of Ayurveda included in Panchakola, Shadushana etc. and attributed dipaniya (stomachic), pachaniya (digestive) properties. It is one of the highly traded species of pharmaceutical industries. To meet the increasing demand has resulted in a decline in their quality. There is now a need to develop a systematic approach for the authentication of herbal plants and to develop well-designed methodologies for their standardization. The present paper deals with the macroscopic and microscopic evaluation of the root of *Piper longum* Linn. along with its micrometric and isolation techniques. Pharmacognostic study of Pippali mula (root of *Piper longum* Linn.) has been carried out as per standard reference procedures. Paranchymatous cells heavily loaded with starch grains, oil globules and fan shaped arrangements of vascular bundle upto centre distinctive the drug from other root morphology. Powder microscopy along with micrometric and isolation techniques would be of immense value in botanical identification and authentication of plant drug and may help us in preventing its adulteration.

**Key words:** Pippali; *Piper longum*; Pharmacognosy; Micrometric.

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## INTRODUCTION

The genus *Piper* (L.) contains more than 700 species grown in tropical and subtropical rain forest. Pippali (Thippali) consists of dried fruits of *Piper longum* L. (Piperaceae) a slender, aromatic, creeping and perennial under shrub, native of the hotter parts of the country and found wild as well as cultivated extensively in Assam, lower hissa of Bengal, ever green forest of Western Ghats, along west coast of Southern States and also recorded from Car Nicobar Islands.<sup>[1]</sup>

The source plant of Pippali mula (*Piper longum* Linn.) is a native of Indo-Malaya region. The Greek name *Peperi*, the Latin *Piper* and the English Pepper were derived from the Sanskrit name Pippali. It grows wild in the tropical rain forests of India, Nepal, Indonesia, Malaysia, Sri Lanka, Rhio, Timor and the Philippines. In India, the plant grows abundantly in Assam, West Bengal, Uttar Pradesh, Madhya Pradesh, Maharashtra, Kerala, Karnataka and Tamil Nadu. It is also cultivated in Andhra Pradesh, Bengal, Chirapunchi area of Assam, Akola-Amravati region of Maharashtra, Anamalai hills of Tamil Nadu, Orissa, Udipi and Mangalore regions of Karnataka.<sup>[2]</sup>

In Ayurveda, Pippali mula (root of *Piper longum* Linn; Piperaceae) is attributed dipaniya (Appetizer) – Pachaniya (digestive) properties and considered as the prime drug to relieve Anaha (Constipation).<sup>[3]</sup> The drug possesses Katu Rasa (pungent taste); Laghu (Light), Ruksha (rough) and Ushna (hot) guna (properties); Madhura vipaka (Specific digestion) and used in various disease conditions i.e. Krimi (parasitic disease), shwasa (dyspnoea), Kshaya (Pulmonary tuberculosis), Pliha roga (spleen disorders), Vishama jvara (intermittent fever), Arsha (piles), Urustambha (stiffness of thigh), Vatavyadhi (Nervous diseases), Nidranasha (Insomnia), Grahani (Dysentery) etc.<sup>[4]</sup> Vangasena, a medieval compendium identified

sedative property of Pippalimula and suggested it with jaggery as anupanna (vehicle). It is excerpted as origin of health (Arogyamoolam) by authors of Siddhabheshaja manimala.<sup>[5]</sup>

Pippali mula have been used as stomachic, thermogenic, aphrodisiac, carminative, expectorant, laxative, digestive and emollient, anti-giardias, anti-amoebic, anti-asthmatic, antiseptic and also active against bacterial diseases. The root is reported to have weak opioid but potent NSAID type of analgesic activity,<sup>[6]</sup> anti oxidant activity,<sup>[7]</sup> anti-microbial<sup>[8]</sup> In folklore practice; root is employed for the treatment of heart disease in East India. In Travancore region an infusion of the root is prescribed after parturition.

As all the parts of the plant *Piper longum* Linn. are medicinally important including root, it was thought worth to study them individually; hence the root part is selected for the scientific investigation for its macro, micro and phytochemical examinations.

## Aims and objectives

To evaluate Pippalimula (*Piper longum* Linn. root) macroscopically and microscopically along with its micrometric and isolation techniques.

## MATERIAL AND METHODS

### Collection of the drug

The healthy, dried Grade – I *P. longum* root was collected from the Paderu district, Andhra Pradesh, where it is cultivated for medicinal purpose in the month of January. The exterior of the root is greyish brown in colour, it was longitudinally wrinkled and having roots and root scars on the surface. Cleaned manually to remove foreign matters and preserved for microscopic sections. Root powder was stored in well closed containers away from the light. (Sample no.: Phm. Raw drug – 6119/13-14)



Figure 1: Pippali mula (*P. longum* root)



Figure 2: Pippali mula (*P. longum* root) powder



Figure 3: TS of *P. longum* root



Figure 4: Stained TS of *P. longum* root

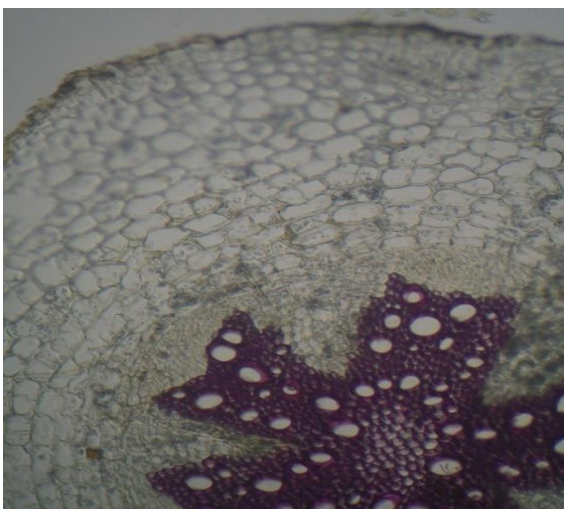


Figure 5: Diagrammatic

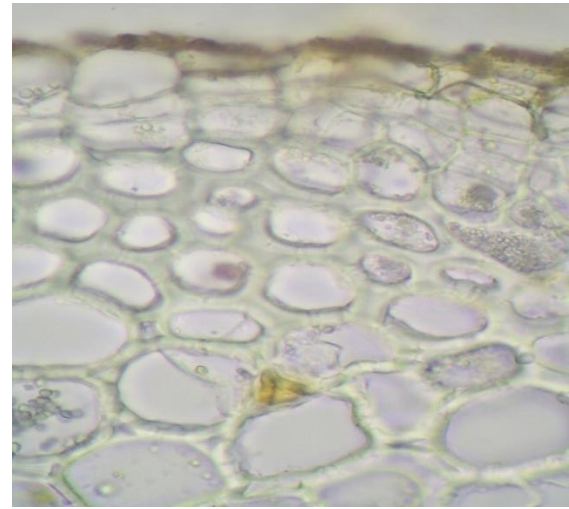


Figure 6: Cortical region



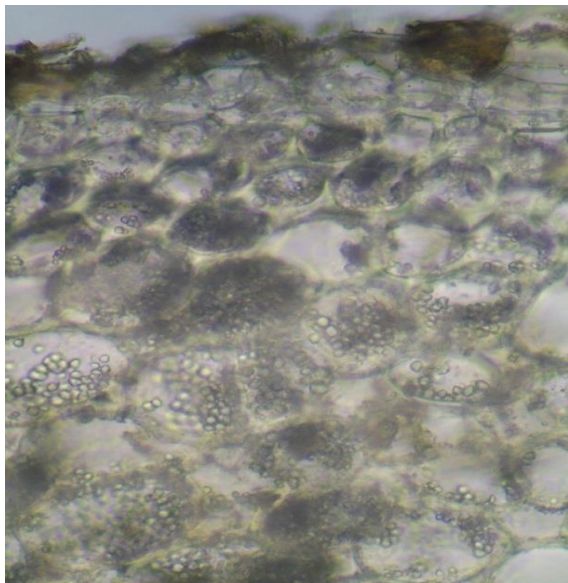


Figure 7: Cells with starch grains and oil globules

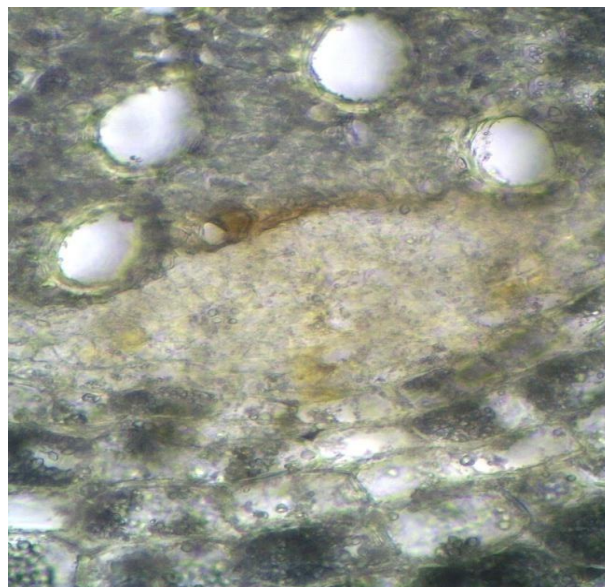


Figure 8: Phloem xylum

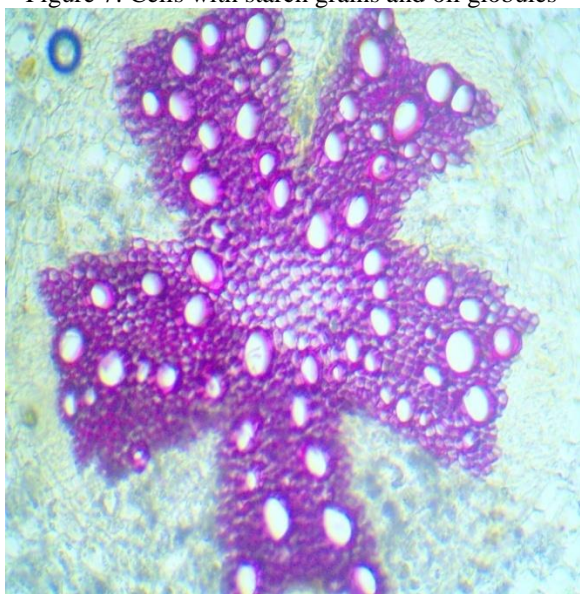


Figure 9: Stealar region

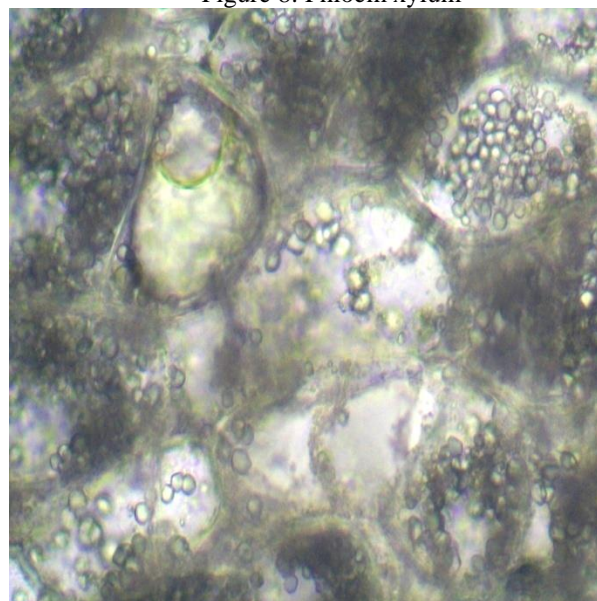


Figure 10: Oil containing cells

**Table1: Measurements of isolated lignified elements of *Piper longum* Linn. Root**

Root	Length	Width
Fibres	589.90	20.82
Vessels	416.40	48.58

**Table 2: Micrometric measurements of *P. longum* root**

Sl. No.	Characters	Measurements (in micrometers)
1.	Compound starch grains	0.47 x 0.43
2.	Simple starch grains	0.3
3.	Pitted vessels	2.33 x 0.53
4.	Annular vessels	2 x 0.6
5.	Rod shaped crystals	0.1 x 0.6
6.	Prismatic crystals	0.7 x 0.5
7.	Cork cells	0.4 x 0.5

**Figure 11: Powder Microscopy of *P. longum* root**



Figure 11.1: Cork in surface view



Figure 11.2: Fibers



Figure 11.3: Group of trechoids

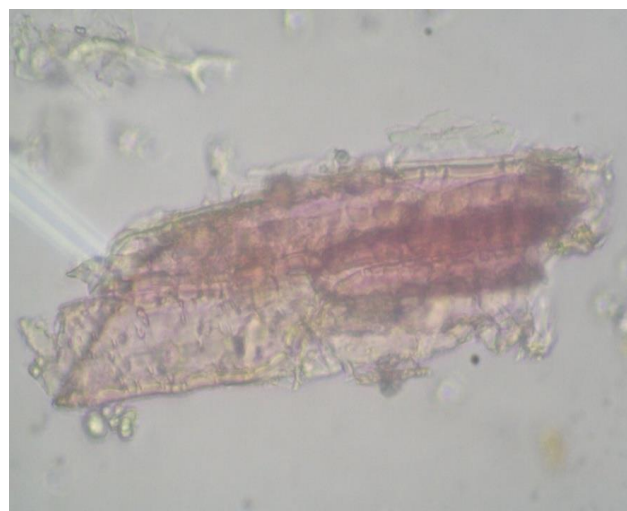


Figure 11.4: Scleroids

**Macroscopic study**

The collected sample was identified and authenticated by studying its characters systematically as per the methods described in the textbooks of pharmacognosy. The specimen was observed as such with naked eyes.

**Organoleptic**

Evaluation of the sample was done by their various characters like, colour, texture, odour, taste etc.<sup>[9]</sup> Free hand sections were taken from

the preserved sample of the root and observed as such under the microscope.

The sections were cleared with chloral hydrate and then stained with phloroglucinol and hydrochloric acid to observe the lignifications of the cell wall if any. The histo-chemical tests were also performed to detect the location of various cell contents by using various reagents. For isolation of the lignified elements the roots were subjected for the Schultz's maceration process.<sup>[10]</sup>



**Figure 12: Micrometry of *P. longum* root**

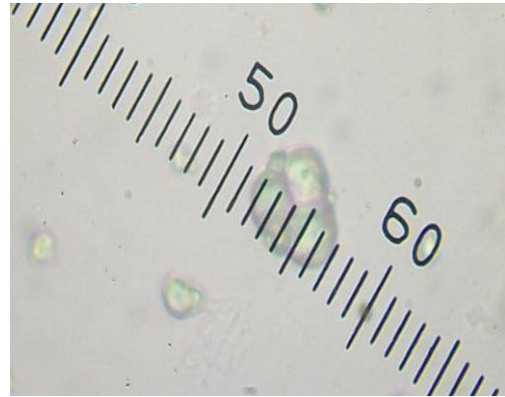
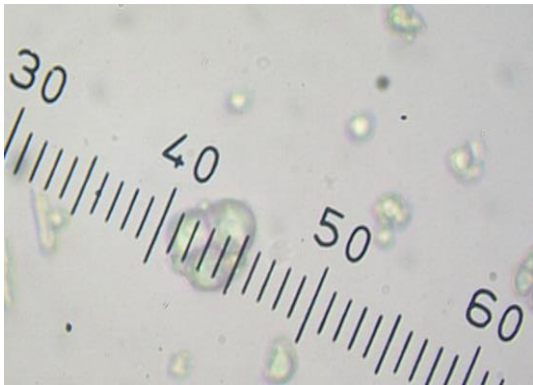


Figure 12.1: Compound Starch grain

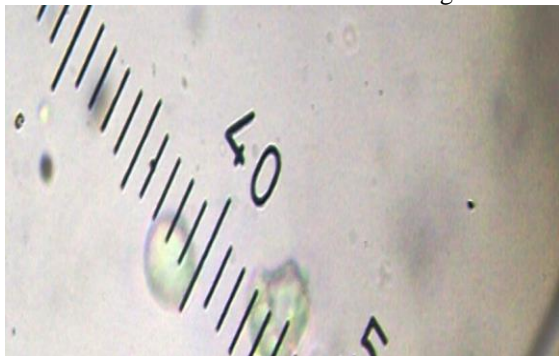


Figure 12.2: Simple starch grains

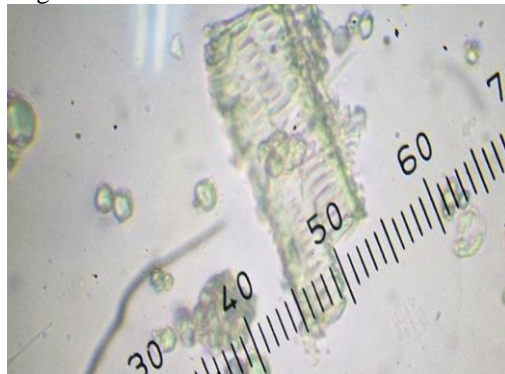


Figure 12.3: Fragment of pitted vessels

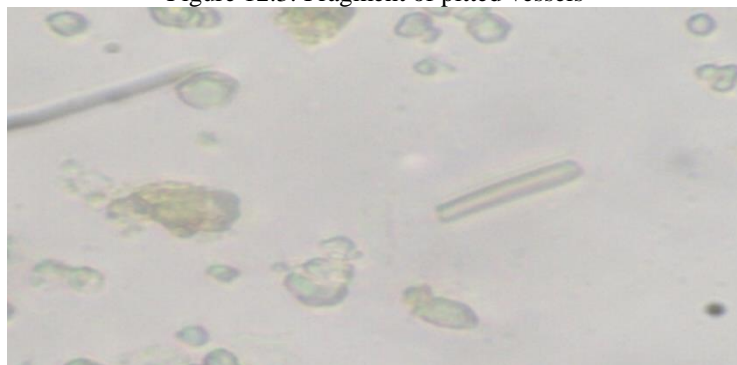


Figure 12.4: Rod shaped starch grains

Photographs of sections and diagnostic characters of the powders were also taken with Canon digital camera and Carl zeiss trinocular microscope.

### Micrometric evaluation

Systemic evaluation of Pharmacognosy of starch grains, pitted vessels etc. followed by micrometry. Using Carl Zeiss binocular microscope attached with camera and preloaded micrometric analysis software the length, breadth of the characters are measured and mean value is taken into consideration.<sup>[11]</sup>

### Isolation technique

Disintegration and isolation of the tissue for the root powder of *Piper longum* Linn. are carried out by boiling the sample with sulphuric acid and maintain at the boiling point for 30 sec. Collected and washed the residue, mounted and examined.<sup>[12]</sup>

### Histochemical

Sample thick sections subjected to Histochemical tests to find starch grains, tannin, calcium etc. by treating with various reagents.<sup>[11]</sup>

## RESULTS

### Morphology

*Piper longum* Linn. is a member of Piperaceae family. The plant is a glabrous perennial under-shrub with erect or sub-scandent nodose stem and slender branches; the latter are often creeping or trailing and rooting below or rarely scandent reaching a few metres height. Leaves are simple, alternate, stipulate, and petiolate or nearly sessile; lower ones broadly ovate, cordate; upper ones oblong, ovate, all entire, smooth, thin with reticulate venation; veins raised beneath. It flowers nearly throughout the year. Inflorescence is spike with unisexual small achlamydeous densely

packed flowers and form very close clusters of small greyish green or darker grey berries. Female spikes with short thick stalk varying from 1.5 to 2.5 cm in length and 0.5 to 0.7 cm in thickness.

A number of geographical varieties are available in different agro climatic regions of India; the most popular being Assam, West Bengal and Nepal varieties. *Piper officinarum* DC; syn. *Chavica officinarum* Miquel., *Piper pepuloides* and *Piper chaba* Hunter. are the other related species are of therapeutic importance.

### Organoleptic characters

Inner surface is cream white, externally it is brownish grey in colour; odour strong, characteristic causing irritation in nose; taste pungent. Fracture: short. (Figures 1 & 2)

### Microscopy

#### Transverse section of root

Diagrammatic section shows outer cork, middle cortex and central stealar region. Detailed transverse section (TS) of a root is almost circular with the regular outline. The outermost tissue cork appears as a narrow strip slightly brown in colour. It consists of 3-5 rows of thin walled and rectangular to slightly tangentially elongated cork cells. The phallogen is not evident in many specimens. (Figure 3 and 4)

The cortex within is fairly wide and paranchyma except for a small thick walled cells. The cortical cells are large sized thin-walled and rounded to oblong with large intercellular space. The cell walls of the peripheral rows are slightly thickened but not lignified. Most of the cells are heavily loaded with spherical or oval shaped, compound starch grains. Many secretory cells filled with yellow large globules are scattered in cortex. (Figure 5 and 6)

**Table 3: Histochemical analysis of powder of *piper longum* Linn. Root**

Reagent	Test for	Color change	Result
Iodine	Starch	Blue	Present
Ferric chloride solution	Tannin	Black	Present
Sudan iii	Oil	Reddish	Present
Phloroglucinol +HCl	Lignin	Pink colour	Present
Phloroglucinol +HCl	Calcium oxalate crystal	Dissolve	Present

Centre pith of the root is occupied by 4-6 wedge shaped radiating strips of vascular tissues called stealar region having their wider ends towards periphery. The cells composing the pith are polygonal, thin walled and full of starch grains. Outside the pith, evenly spaced six groups of primary xylum bundles are present. In each vascular strip the xylum is composed of xylum vessels and xylum parenchyma surrounded with woody fibres. Wider end of xylum is crowned with a hemispherical strip of phloem. These xylum vessels are together arranged in 3-4 radial rows. Few thick walled xylum parenchyma cells are there along with wood fibres. (Figure 8 and figure 9)

A strip of cambium consisting one or two rows of narrow thin walled rectangular cell is present between xylum and phloem. The phloem is composed of many sieve tubes with their companion cells that can be distinctly made out towards the inner region of the phloem and small thin walled polygonal phloem parenchyma cells. The cells at the outer convex side are slightly compressed and appear tangentially elongated. One or two groups of stone cells are present at the peripheral region of the phloem. The outer border of the phloem is limited by a row of pericyclic cells found just inner to the endodermis. (Figure 9 and figure 10)

Medullary rays are four to six broad wedges shaped medullary rays with their wider ends at the periphery and alternating with the radiating bands of vascular tissue. Each ray is having ten to fifteen cells in width and extended from pith upto endodermis.

The ray cells are all thin walled cubical to slightly radially elongated and arranged in regular radial rows. Most of the cells are heavily loaded with starch grains similar to those present in the cortex. Some cells contain minute crystals of calcium oxalate and a few cells have oil globules. In some specimens narrow strips of inter fascicular cambial cells are found connecting the fascicular cambia present in the vascular strip. Large quantities of compound starch grains are specific to *Piper longum* Linn. (Figure 9)

### Powder Microscopy

#### Organoleptic characters

Course, fibrous, brownish white coloured with pungent and characteristics odour. (Figure 2) Diagnostic characteristics of root powder showed that cork in surface view, parenchyma cells with simple and compound starch grains, prismatic crystals and rod shaped crystals of calcium oxalate, annular and border pitted vessels, fragments of lignified fibres and sclerides distributed throughout the powder. (Figure 11)

Measurement of isolated lignified xylems and fibres of *Piper longum* Linn. root was carried out. Result was reported in tabular manner. (Table 1; Figure 12)

The evaluations of micrometry values are constant and stable in different environmental conditions. The values were scientifically studied and measured under the microscope by trial and error method. The mean values are taken into consideration. The obtained



measurements are prosecuted in tabular manner. (Table 2)

Histochemical tests of the plant shows presence of Starch, Tanin etc. The thick sections were treated with various reagents i.e., ferric chloride for tannin, Iodine for starch etc, the results were depicted in the table. (Table 3)

### DISCUSSION AND CONCLUSION

Morphology of the root reveals that externally it is brownish grey in colour; odour strong, characteristic, causing irritation in nose while in raw form. TS of the root show cork with tannin, fan shaped arrangements of vascular bundle upto centre is the most important striking character of the Pippalimula without forming any pith. It was distinctive from other root morphology. Oil globules and starch grains are distributed throughout the root sections.

Powder microscopy of the root showed pitted, scleriform and spiral vessels, simple and compound starch grains with tannin which are the specific characters of *Piper longum* Linn. root. (Figure 10)

Isolated fibres and vessels give accurate information regarding powder characters while, micrometric evaluation can be utilised for specific values for authentication of the drug and avoidance of the adulterants. (Figure 11)

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