

## Comparative Preliminary Foliar Phytochemical Screening of *Diospyros malabarica* (Desr.) Kostel and *Diospyros lanceifolia* Roxb.

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### ABSTRACT

Phytochemical screening is one of the necessary steps to find out the chemical constituents which lead the isolation of compounds. The leaf extract of *Diospyros malabarica* and *Diospyros lanceifolia* was performed for the biologically active secondary metabolites: alkaloids, protein and amino acids, flavanoids, steroids, triterpenoids, tannins, anthocyanin, saponin glycoside, phenol, lipid, gelatin, starch, carbohydrate, reducing and non reducing sugars. The leaf extract which is generally used as a folk medicine due to the presence of steroid, triterpenoids, alkaloid and tannins.

**Key words:** Phytochemical screening, *Diospyros malabarica* (Desr.) Kostel, *Diospyros lanceifolia* Roxb.

### INTRODUCTION

Plants are natural sources of producing wide number of phytoconstituents in a most efficient way. Since the middle of the 19<sup>th</sup> century, different bioactive phytoconstituents have been isolated and characterized. Many of these are used as active ingredients of the modern medicine, or as the lead compounds for new drug discovery. Several plants derive medicines which are rich in phenolic compounds<sup>1</sup>, such as those used in the protection against coronary heart diseases and carcinogenesis<sup>2</sup>.

*Diospyros* L. of Ebenaceae family is characterized by tree or shrubby habit with glabrous branches. Leaves are usually alternate or sometimes sub-opposite or opposite, sessile, simple, entire, exstipulate, narrow elliptic or lanceolate to oblong-acuminate or obtuse, glabrous or pubescent, often anisophyllous. Inflorescence axillary, cymose, fasciculate, pseudo-racemose, bracteates. Flowers usually unisexual rarely hermaphroditic, regular, 3-7 merous, dioecious very rarely polygamous. From old days different *Diospyros* sp are known for their medicinal uses. In many traditional medicinal systems of the world, a number of *Diospyros* plants are used as medicinal agents against various diseases. All parts of these plants are used for medicinal purposes such as the leaves used for lumbago, fruits are carminative, astringent, and cure biliousness, the seed are sedative and the bark is bitter, astringent and febrifuge<sup>3</sup>.

*Diospyros* species are a rich source of biologically active compounds and almost all parts of plants in this genus have been used as traditional medicine<sup>4</sup>. Plants in this genus are well documented, and are reported to contain naphthoquinones, including 7-methyljuglone, diospyrin, isodiospyrin<sup>5</sup> and triterpenes of the lupine series. The latter have been found to exhibit ichthyotoxic, antimicrobial and antitumor activities<sup>6,7</sup>. Naphthoquinones produced by this

genus are usually in the form of dimers<sup>7</sup>. Other biologically active compounds that have been reported from *Diospyros* species are coumarin, flavonoids and other phenolic compounds<sup>4</sup>. Thus, *Diospyros* sp in traditional medicinal system of the world are used as antifungal and (a) for internal hemorrhage and bedwetting in children (b) woman's medicine, for insomnia and hiccough, (c) anti-hypertensive (d) dysphonia (e) vermicide and vermifuge (f) sedative (g) antifebrile (h) promotes secretions (i) astringent and (j) bactericidal<sup>8,9</sup>. Phytochemical studies have been previously carried out on many *Diospyros* species and have revealed the widespread presence of naphthoquinones and naphthalene derivatives, dimeric naphthoquinones and lupine triterpenes<sup>10</sup>. Not much has been elicited on the qualitative and quantitative foliar chemical estimation of *Diospyros* and hence the present study is an attempt to evaluate foliar chemical constituents of the selected species.

### MATERIALS AND METHODS:

#### *Collection and preparation of plant materials*

Flowering twigs of *D. malabarica* (Desr.) Kostel and *D. lanceifolia* Roxb. were collected from various localities of Kamrup district of Assam. Voucher specimens were processed following standard herbarium techniques<sup>11</sup> and were identified with the help of relevant literatures<sup>12, 13</sup> and previously identified specimens at GUBH, ASSAM, CAL and also with images of herbarium specimens of online databases of various herbaria like K, JSTOR and EOL. The collected samples were washed thoroughly, sliced and oven dried at 60°C until they were completely dried and get constant weight. The dried slices were then powdered and kept at 4°C for further analysis. The plant powder was used directly for the preparation of the crude extract in different solvents as per necessity of the experiment.

Table 1: Table showing biochemical analysis of samples

Sl no.	Phytochemical constituents	Name of the tests	Plant extract	
			<i>D.malabarica</i>	<i>D.lanceifolia</i>
1	Carbohydrate	Benedict test	+	+
		Fehling test	-	+
2	Starch	Iodine test	-	-
		Lugol Iodine test	-	-
3	Lipid	Emulsification test	-	-
		Solubility test	-	-
4	Protein & Amino acid	Ninhydrin test	-	-
		Biuret test	-	-
5	Flavanoid	NaOH test	-	-
6	Alkaloid	Mayer's test	+	+
		Wagner's test	+	+
7	Phenol	FeCl <sub>3</sub> test	+	+
8	Gelatin	Solubility test	-	+
		NA plating test	-	-
9	Steroid	Salkowski test	+	+
10	Triterpenoid	Salkowski test	-	-
		Hirshonn test	-	-
11	Saponin Glycoside	Froth formation test	+	+
12	Tannin	Lead acetate test	+	+
13	Anthocyanin	H <sub>2</sub> SO <sub>4</sub> test	-	-
14	Reducing and Non-Reducing sugar	Benedict test	+	+

Table 2: Table showing quantitative estimation of phytochemicals

Sl no	Chemical constituent	Standard	Methanolic extract (µg)	
			<i>D.malabarica</i>	<i>D.lanceifolia</i>
1	Carbohydrate	Glucose solution	67.65	54.36
2	Alkaloid	Atropine	53.32	17.68
3	Phenol	Gallic acid	1.58	19.85
4	Steroid	Atropine	26.36	28.14
5	Tannin	Folins' reagent	9.55	20.59
6	Reducing sugar	Glucose solution	31.40	65.77
7	Non reducing sugar	Glucose solution	49.53	63.22

#### Phytochemical Analysis

Chemical analysis was done on moisture free basis to estimate the phytochemicals by using standard procedure to identify the constituents<sup>14,15,16</sup>.

#### Test For Carbohydrates:

**Fehling's Test:** To the extract, equal quantities of fehling's solution A and B were added and on heating, formation of a brick red precipitate indicates the presence of carbohydrates.

**Benedict's Test:** To 5ml of Benedict's reagent, extract was added and boiled for two minute and cooled. Formation of a red precipitate showed the presence of carbohydrates.

#### Test For Starch

**Iodine Test:** As the sample solution is dark in colour so, to the solution, 2-3 drops of freshly prepared iodine solution was added and the formation of blue-black colour solution indicates the presence of starch.

**Lugol Iodine Test:** It is also known as iodine-KI reagent and composed of aqueous Iodine solution in presence of KI. Few drops of iodine-KI reagent was added to the aqueous solution of starch, which produces deep blue to bluish black colouration due to presence of amylase. The

colour developed disappears on warming and reappears on cooling.

#### Test For Lipid

**Emulsification Test:** If emulsifiers like bile salts, tween or soap solution was mixed with lipids and water; the lipids broken down into smaller fragments, which remain suspended for long periods of time in water.

**Solubility Test:** Lipids were insoluble in polar solvents like water and soluble in nonpolar solvents like petroleum ether, benzene and mineral oil.

#### Test For Proteins And Amino Acids

**Biuret Test:** To the aqueous solution of protein in hot water, few drops of Biuret reagent ( KOH, CuSO<sub>4</sub> and Sodium potassium tartarate) were added, which turns blue reagent to pink or violet. In laboratory it was done by adding 1 ml of 4 % copper sulphate (CuSO<sub>4</sub>) solution to the alkaline aqueous protein solution. At least one peptide linkage was necessary for this test: individual amino acids do not produce pink or violet colouration.

**Ninhydrin Test:** The ninhydrin test was used to detect the presence of alpha-amino acids and proteins containing free amino groups. Protein solution when heated with ninhydrin molecule it gives characteristic deep blue or pale

Table 3: Table showing percentage transmittance and absorbance of different phytochemicals from the crude extract

Sl no.	Phytochemical constituents	Solvent	% transmittance		Absorbance (A)	
			<i>D. malabarica</i>	<i>D. lanceifolia</i>	<i>D. malabarica</i>	<i>D. lanceifolia</i>
1	Carbohydrate	Benedict solution	45.64%	32.35%	0.34	0.49
2	Alkaloid	Iodine solution	82.32%	92.92%	0.08	0.03
3	Phenol	FeCl <sub>3</sub> solution	80.15%	94.28%	0.09	0.03
4	Steroid	Sulphuric acid	63.03%	73.64%	0.20	0.13
5	Tannin	FeCl <sub>3</sub> solution	84.47%	89.04%	0.07	0.05
		Lead acetate solution	78.09%	94.71%	0.11	0.02
6	Reducing sugar	Benedict solution	65.24%	68.59%	0.18	0.16
7	Non-Reducing Sugar	Benedict solution	93.42%	50.46%	0.02	0.29

yellow colour due to formation of complex between two ninhydrin molecule and nitrogen of free amino acid. Ninhydrin reagent is 0.1 % w/v solution of ninhydrin in n-butanol.

#### Test For Flavanoid

Sodium hydroxide (NaOH) Test: 5gm was dissolved in water, warmed and filtered. 10% aqueous NaOH added to 2ml of solution. This produces a yellow colour. A change in the colour from yellow to colourless on addition of dilute Hcl indicates presence of flavanoid.

#### Test For Alkaloid

Few mg of the residue of each extract was taken separately in 5 ml of 1.5 % v/v hydrochloric acid and filtered. These filtrates were then used for alkaloid detection.

Mayer's Reagent: 1.36 g of mercuric chloride was dissolved in 60 ml water and 5 g of potassium iodide dissolved in 10ml of distilled water, solution was mixed and diluted to make up volume 100 ml. To a little of each extract taken in dilute hydrochloric acid in a watch glass, few drops of the reagent was added, formation of cream colored precipitate shows the presence of alkaloids.

Wagner's Reagent: 1.27 g of iodine and 2 g of potassium iodide were dissolved in 5 ml of water and the solution was diluted to 100 ml with water. When few drops of this reagent were added to the test filtrate, a brown color precipitate was formed indicating the presence of alkaloids.

#### Test For Phenol

Ferric chloride (FeCl<sub>3</sub>) Test: To the extract, few drops of 10 % aqueous ferric chloride were added. Appearance of blue or green color indicates the presence of phenols.

#### Test For Gelatin

Solubility Test: Soluble in hot water as well as cold water.

#### Test For Steroid

Salkowski Test: To the plant extract few drops of sulphuric acid were added, which will create a layer of red colour at lower side and will indicate the presence of steroid.

#### Test For Triterpenoids

Salkowski Test: To the plant extract few drops of sulphuric acid were added, and formation of yellow coloured lower layer will indicate the presence of triterpenoid.

Hirshonn reaction: When a substance was heated with trichloro acetic acid, red to purple color was observed as triterpenes on addition of saturated trichloro acetic acid solution forms coloured precipitate.

#### Test For Saponin Glycosides

Foam Test: A few mg of the test residue was taken in a test tube and shaken vigorously with small amount of sodium bicarbonate and water. If stable, characteristic honeycomb like froth is obtained, saponins are present.

#### Test For Tannin

The test residue of each extract was taken separately in water, warmed and filtered. Tests were carried out with the filtrate using following reagent-

Ferric Chloride (FeCl<sub>3</sub>) Test: A 5 % solution of ferric chloride in 90 % alcohol was prepared. Few drops of this solution was added to a little of the above filtrate. If dark green or deep blue color is obtained, tannins are present.

Lead Acetate (C<sub>4</sub>H<sub>6</sub>O<sub>4</sub>Pb) Test: A 10 % w/v solution of basic lead acetate in distilled water was added to the test filtrate. If precipitate is obtained, tannins are present.

#### Test For Anthocyanin

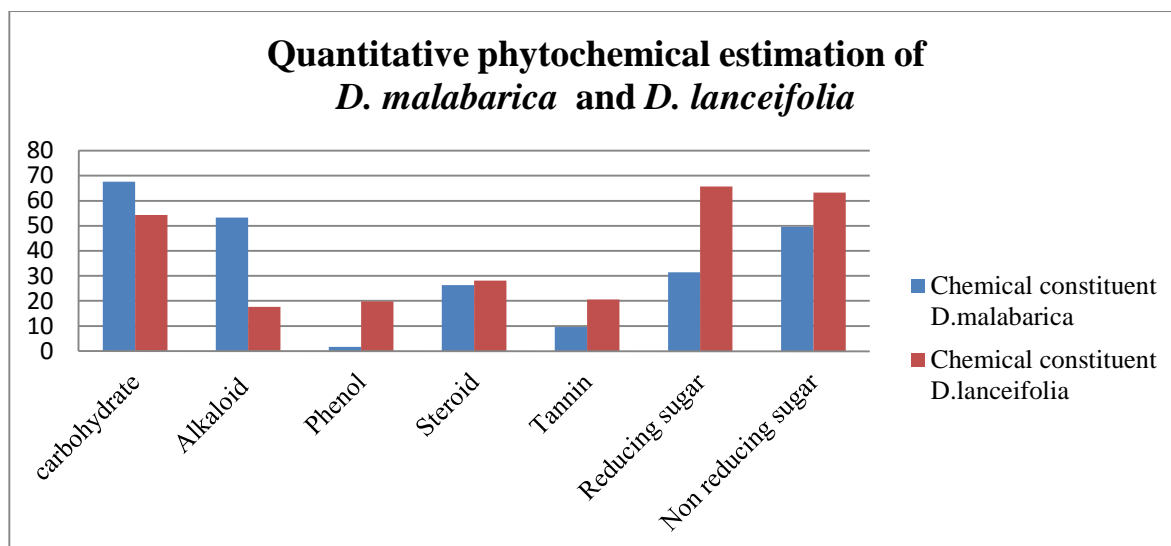
Sodium hydroxide (NaOH) Test: To the solution, 10% sodium hydroxide was added, the development of blue color shows the presence of anthocyanins.

Sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) Test: To the substance conc. sulphuric acid was added, the development of yellowish orange color confirms the presence of anthocyanins.

#### Test For Reducing And Non-Reducing Sugar

Benedict Test: All monosaccharides and most disaccharides (except sucrose) will reduce copper sulphate (CuSO<sub>4</sub>), producing a precipitate of copper oxide (CuO) on heating, so they are called reducing sugars. Benedict's reagent is an aqueous solution of CuSO<sub>4</sub>, Na<sub>2</sub>CO<sub>3</sub> and sodium citrate. To approximately 2 ml of test solution add an equal quantity of Benedict's reagent. Shake, and heat for a few minutes at 95°C in a water bath. A precipitate indicates reducing sugar. The colour and density of the precipitate gives an indication of the amount of reducing sugar present, so this test is semi-quantitative. The original pale blue colour means no reducing sugar, a green precipitate means relatively little sugar; a brown or red precipitate means progressively more sugar is present.

Sucrose is called a non-reducing sugar because it does not reduce copper sulphate, so there is no direct test for sucrose. However, if it is first hydrolysed (broken down) to its constituent monosaccharide's (glucose and fructose), it will then give a positive Benedict's test. So sucrose is the only sugar that will give a negative Benedict's test before hydrolysis and a positive test afterwards. Using a



The graphical representation of the quantitative estimation of the phytochemicals

separate sample, boil the test solution with dilute hydrochloric acid for a few minutes to hydrolyse the glycosidic bond. Neutralise the solution by gently adding small amounts of solid sodium hydrogen carbonate until it stops fizzing, then shake, and heat for a few minutes at 95°C in a water bath. A precipitate indicates non reducing sugar.

## RESULTS

### Comparative preliminary phytochemical screening

Different phytochemical screening were performed to know the compounds present in the leaf sample of *D. lanceifolia* and *D. malabarica* which are presented in tabular forms (Table 1). The phytochemical analysis was performed to confirm the protein content of the leaf extract and to determine if other any other compounds like carbohydrate, starch, lipid, flavanoid, alkaloid, phenol, gelatin, steroid, triterpenoid, saponin glycoside, tannin, anthocyanin, reducing and non-reducing sugar etc.<sup>17</sup>

*D. malabarica*: Various phytochemicals like carbohydrate, alkaloid, phenol, steroid, saponin glycoside, tannin, anthocyanin, reducing and non-reducing sugar were proved to be present by performing various activity tests. But some other chemical constituents like starch, lipid, protein & amino acid, flavanoid, gelatin, triterpenoid were absent.

*D. lanceifolia*: Various phytochemicals like carbohydrate, alkaloid, phenol, gelatin, steroid, saponin glycoside, tannin, anthocyanin, reducing and non-reducing sugar were proved to be present by performing various activity tests. But some other chemical constituents like starch, lipid, protein & amino acid, flavanoid, triterpenoid were absent.

The qualitative phytochemical analysis showed that the leaf extract *D. lanceifolia* contain more chemical constituents (9 numbers) as compared to *D. malabarica* (8 numbers).

### Comparative quantitative phytochemical estimation

The quantitative phytochemical analyses of the leaf extracts along with standard were assessed with the help of

UV-VIS spectrophotometer (540nm) and the comparisons between the various concentrations were calculated. Only those chemical constituents which qualitatively found to be present were estimated quantitatively along with % transmittance and absorbance for future reference.

*D. malabarica*: The various phytochemicals like carbohydrate, phenol, steroid, saponin glycoside, tannin, anthocyanin, reducing and non-reducing sugar were estimated by performing various activity tests in which the quantity of carbohydrate was the highest (67.65µg) and phenol was least (1.58µg), while the % transmittance of non-reducing sugar were highest (93.42%) followed by tannin (84.47%) and the % transmittance of carbohydrate (45.64%) was found to be lowest of all the chemicals estimated.

*D. lanceifolia*: The various phytochemicals like carbohydrate, alkaloid, phenol, gelatin, steroid, saponin glycoside, tannin, anthocyanin, reducing and non-reducing sugar were estimated in which the quantity of reducing sugar (65.77µg) was highest and alkaloid (17.68µg) was lowest while % transmittance of tannin (94.71%) was highest followed by phenol (94.28%) and % transmittance of carbohydrate (32.35%) was lowest of all the chemicals estimated.

In the present study it was found that the leaf extract of *D. lanceifolia* contain a great number of tannin, alkaloid and phenolic compound as compared to *D. malabarica*. Whereas the leaf extract of *D. malabarica* contain non-reducing sugar in great extent.

From the above table (Table 3) it was found that *D. lanceifolia*. contain more % transmittance and absorbance of the phytochemical constituents of leaf samples as compared to *D. malabarica*.

## DISCUSSION

Phytochemical screening of methanolic extracts of *D. malabarica* and *D. lanceifolia* fractions showed the presence of various secondary metabolites like carbohydrate, alkaloid, phenol, gelatin, steroid, saponin glycoside, tannin, anthocyanin, reducing and non-reducing

sugar. The medicinal value of these two plants can be correlated due to the presence of various bio-active chemical constituents. Crude methanolic extract of the plant showed the presence of polar and non polar phytoconstituents. The leaves of these plants are used as a folk medicine is due to the presence of bio-active phytoconstituents which need further research. The literature revealed that the genus *Diospyros* L. have pesticide and biological activities<sup>18,19</sup>. The specific activity of the *Diospyros* L. may be attributed to the presence of proteins, triterpenoids, steroids and other secondary metabolites which need further investigation as well as isolation for being a beneficial one in the field of pharmacology and medical biology.

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