

PRELIMINARY PHYTOCHEMICAL AND HISTOCHEMICAL INVESTIGATION ON KIGELIA PINNATA DC.,

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ABSTRACT

Kigelia pinnata D.C. the mid sized ornamental tree of the Bignoniaceae has been studied by preliminary phytochemical and histochemical analysis. Several local names are availability to this plants based on their country. They are called worsboom in Africa and sauge tree in America. The tree is 25 meters in high with a dense rounded crown bark grey. Data gathered on solvent extraction and preliminary phytochemical method suggested that the presence of glycosides, flavonoides, tannin and alkaloids in leaf tissue. Anatomical and histochemical investigation offered some clues on the localization of certain specific metabolites. This paper revealed preliminary phytochemical constituents of *Kigelia pinnta* D.C., by phytochemical and histochemical investigation.

KEY WORDS: *Kigelia pinnta* DC, phytochemical constituents, solvent extraction, histochemical analysis.

INTRODUCTION

The history of herbal medicine is as old as human civilization .Documents available reveals that plants were used medicinally in China, India, Egypt and Greece long before the Christian era. Recent investigations indicate that plants have anticancer activities (1). ***Kigelia pinnata* D.C.**, a member of Bignoniaceae has hidden potential. Several plants of this family are reported to have medicinal properties. Documented species distribution shows that this tree is a native tree in African continent .Exotic to India, Australia, U.S.A. Available literature clearly shows that this plant has many traditional uses. The plant can grow in open land,wilt,high rainfall, savanna (2). The plant has medicinal properties not only because of its perceived characteristics such as bitterness, astringent, taste, smell but also because of forces that it seems to emit in connection with its location ,orientation , and association with other plants .It has several medicinal properties such as antimicrobial (3), anti-neoplastic ,analgesic , anti-inflammatory , anti-malarial (4) and anti-protozoal (5).

MATERIALS AND METHODS

Extraction preparation

The plant leaves are shade dried and powered .A weighted quantity of the plant leaf powder was extracted with different solvent separately such as Petroleum ether, Chloroform, Benzene, and Water by cold maceration techniques. Identification of the various phytochemical constituents were carried out adopting the procedures described by Horborne (6), Krishnamurthy (7) in accordance with the general plan of the experiment.

PRELIMINARY PHYTOCHEMICAL ANALYSIS

The Petroleum ether, Chloroform, Benzene, and Water extract were subjected to chemical tests to identify chemical constituents of the plant.

DETECTION OF CARBOHYDRATES

Dissolved minimum amount of extracts in 5ml of distilled water and filtered. The filtrate subjected to the following tests shall be detected for the presence of carbohydrates. A response otherwise indicates the absence of carbohydrates.

Fehling's Test

Small portion of the filtrates treated with equal volume of Fehling's solution A and B and then heated is expected to form a brick red precipitate for confirming the presence of reducing sugar.

Benedict's test

If a small portion of the filtrates were treated with equal volume of Benedict's reagent forms yellow precipitate the response of the extract is said to indicate the presence of reducing sugar.

Test for Starch

An instant bluish black coloration develops when starch is present in the small amount of extract treated with dilute iodine solution. Absence of color shows the absence of starch.

TEST FOR PROTEINS AND AMINO ACIDS

Even small quantities amino acids present in the alcoholic extract if dissolved in few ml of distilled water and subjected to Ninhydrin test and Biuret can be detected at ease.

Ninhydrin Test

When ethanol and aqueous extract are treated with ninhydrin reagent (0.1% solution) and boiled a purple color can be observed for a positive result.

Biuret Test

To a portion of the above prepared extracts, if equal volumes of 5% w/v sodium hydroxide and 4 drops of w/v copper sulfate solution were added, a color change is expected. In case if amino acids are absent the violet color was not be formed.

Test with Tannic Acid

To the above prepared extracts, if one add with 10% tannic acid solution a white precipitate was appear if proteins are present.

TESTS FOR ALKALOIDS

Small amount of the solvent free ethanolic and aqueous extracts are separately stirred with a few ml of dilute HCL and filtered. Filtrates are tested with various alcoholic reagents.

Dragendorff's Test

To small quantity of the extracts, Dragendorff's reagent was added to produce a orange brown precipitate for the detection of alkaloids.

Mayer's Test (8)

To small quantity of the extracts, Mayer's reagent was added. Presence of creamy white precipitate indicates the presence of alkaloids.

TEST FOR FLAVANOIDS

A different portion of the extract can be separately dissolved in ethanol and then subjected to the following test.

Flavonoids (9)

If a portion of the extract or a powdered plant sample is heated with 10ml of ethyl acetate over a steam bath for 3 minutes and filtered, the filtrate of 4 ml is shaken thoroughly with 1ml of dilute ammonia solution, a yellow coloration develops at instance when flavonoids are present.

Ferric Chloride Tests (10)

To a small quantity of the ethanolic extract when a few drops of neutral ferric chloride can be added to end up in Blackish red color. Color change indicates the presence of flavanoids.

Alkaline Reagent Test

Few ml of methanolic extract treated with few drops of neutral ferric chloride forms bluish red colour to indicate the presence of flavonoids.

Mineral oil reduction test

The yellow coloration resulting from the mixing of the extract and boric acid indicates the presence of flavonoids .

Flavones

If an extract gives a yellow color when added to sodium hydroxide solution the presence of flavones can be suspected.

TEST FOR TERPENOIDS

5 ml of each extract was mixed in 2 ml of chloroform and 3ml of conc.H₂ SO₄ was carefully added to form a reddish brown color layer at the inter phase if terpenoids are to be detected.

TEST FOR TANNINS

For this purpose the extracts are dissolved in water and filtered. The filtrates can be subjected to test with the following reagents.

Mace test (11) About 0.5 g of powdered sample was boiled in 20 ml of distilled water in test tube and then filtered. A brownish green or bluish black coloration that resulting with the addition of few drops 0.1% ferric chloride indicates the presence of tannin.

Ferric Chloride Test

Few ml of the filtrates were treated with 5% ferric chloride solution. A bluish black color observed indicates the presence of tannins.

Reduction with CuSO₄

Few ml of the filtrates were treated with 5% CuSO₄ solution. Bluish green precipitates produced in ethanolic and aqueous extracts indicate the presence of tannins.

Potassium Ferric Cyanide Test

Few milliliters of the filtrate when treated with strong potassium ferric cyanide followed by ammonia will develop into a characteristic red color if tannin is present in the extract.

TEST FOR SAPONINS (11)

The extracts were diluted with 20 ml of distilled water and agitated in a graduated cylinder for 15 minutes. Foam formation is the expected positive result if not the extract is said to lack saponin.

HISTOCHEMICAL INVESTIGATION

The fresh, healthy sample (leaf, petiole) were cut and removed from the plant. Thin section of leaf and petiole treated with various reagent and observed the colour formation in specific cells of the tissues.

RESULT

Kigelia pinnata D.C., the tree member of the Bignoniaceae is a genus academic, ornamental and medicinal importance. Despite the interest in the genus, *Kigelia* remains a rare plant to be spotted in field because of the problem it faces in completing its seed cycle. The preliminary phytochemical tests are conducted as mentioned above and result provided (figure 1 & table-1). Histochemical study is conducted and result shown (figure-2, table-2 & figure-3, table-3).

DISCUSSION

Phytochemical screening showed that the leaves and petioles were rich in chemical constituents. Alkaloids, saponins, anthraquinones, glycosides, phenolics, terpenoids and flavonoids have been documented in this study. These principles have been known for many years to exhibit biological activity, such as effects on the central nervous system, and antibacterial, antitumour, and anthelmintic activity (6)

The plants studied here can be seen as a potential source of useful drugs. Solvent extraction and preliminary phytochemical evaluation of crude extract from the leaves of the plant speaks the traditional therapeutic significance of the species. The study shows that the leaves of *Kigelia pinnata* Dc is a house repository of alkaloids, flavones, flavonoids, glycosides and tannin. Further studies are recommended on these plants in order to isolate, identify, characterize and elucidate. Therefore the plant in this study is validated as medicinal.

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Fig 1. Phytochemical screening of solvent extracts from *Kigelia pinnata* Dc

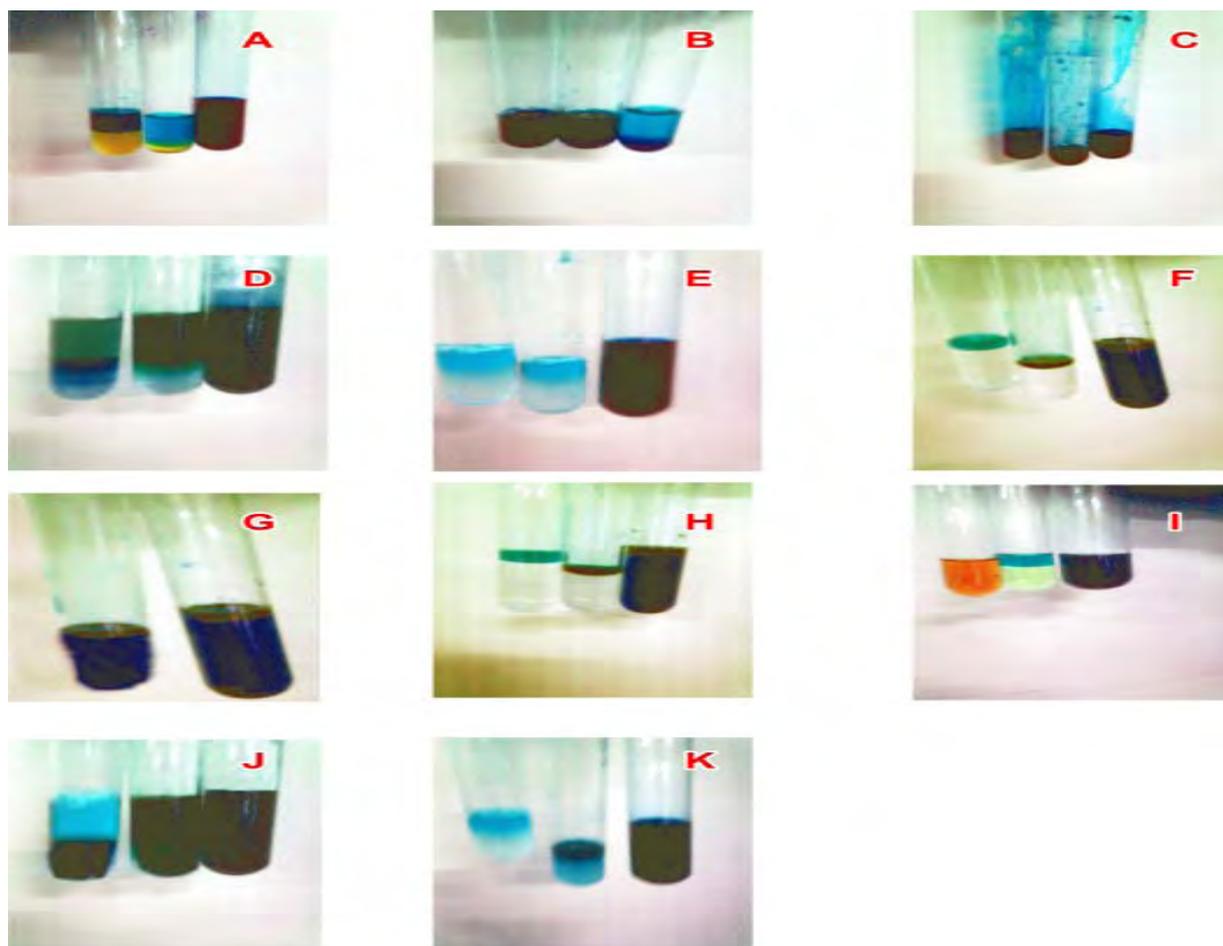


Fig 2. Localization of specific phytochemicals in different cell types of the leaf in *Kigeila pinnata* Dc

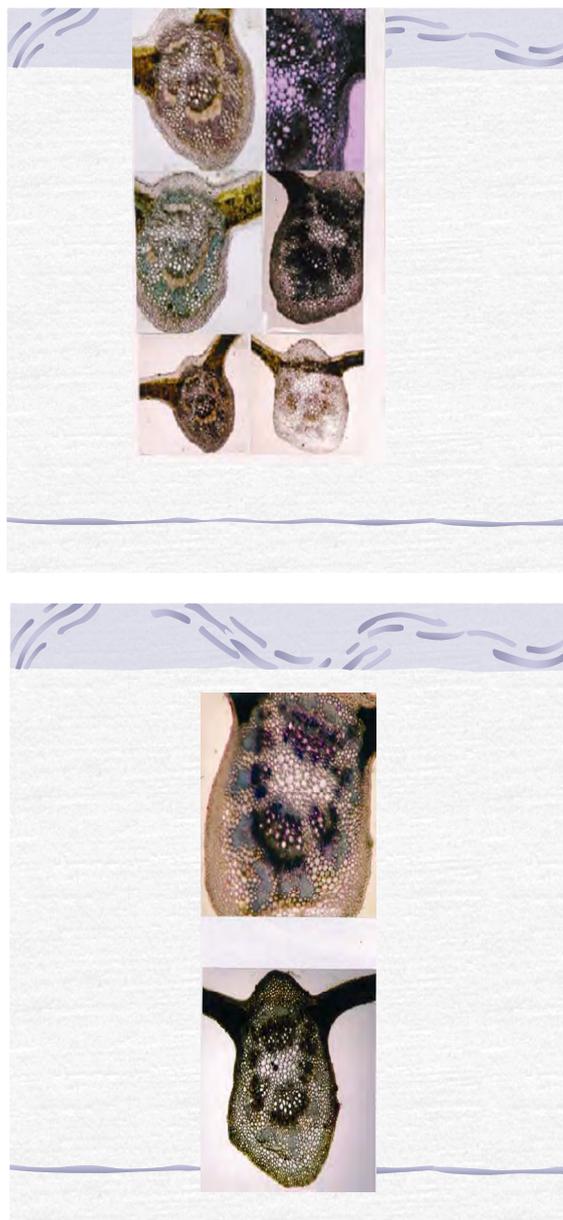


Fig 3. Localization of specific phytochemicals in different cell types of the petiole in *Kigelia pinnata* DC .

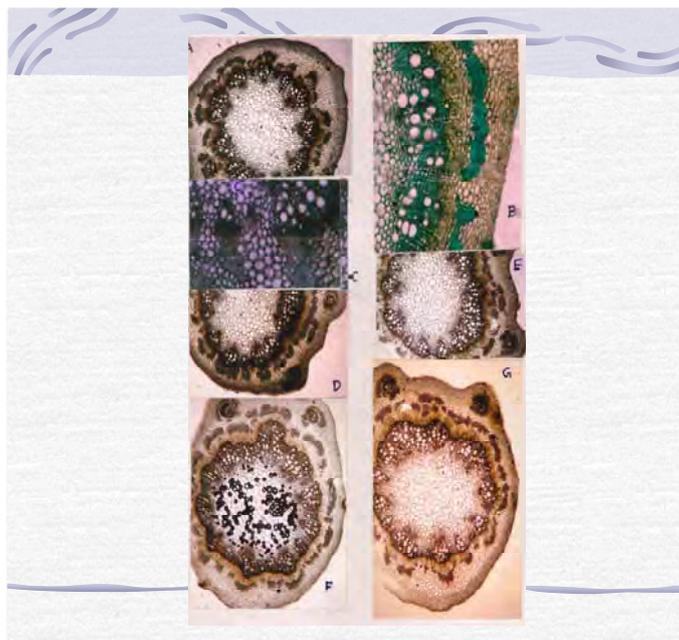


Table 1. Phytochemical screening of solvent extracts from *Kigelia pinnata*

Order	Plant constituent	Test	Solvent types		
			Petroleum Ether	Chloroform	Water
A	Carbohydrates	Fehling	-	-	-
		Bendict	-	-	-
B	Protein	Biuret	-	-	-
		Tannic acid	-	-	-
C	Alkaloid	Mayer	+	+	+
		Dragendorff	+	+	+
D	Terpenoids	Salkowski	+	-	-
E	Flavones	-	+	-	-
F	Flavinoids	Sofowara	-	-	+
		Alkaline reagent	-	-	+
K	Phlobattanin	-	-	+	
G	Tannins	Mace	+	+	+
		FeCl ₃	+	+	+
		CuSo ₄	+	+	+
		Potassium ferricyanide	+	+	+
H	Saponin	-	+	+	
I	Iridioid Glycoside	Trim Hill	+	-	-
J	Starch	Anthrone	+	+	+

+ indicates the presence of the compound

Table 2. Attempt towards localization of specific phytochemicals in different cell types of the leaf in *Kigeila pinnata* DC

S.No	Tissue Type	A	B	C	D	E	F	G	H
1	Upper Epidermis	+	-	-	-	-	-	-	-
2	SubEpidermis	+	-	+	+	-	-	-	-
3	Palisade(Lamina)	+	+	-	+	-	+	-	-
4	Spongy Parenchyma (Lamina)	+	+	+	-	-	+	+	+
5	Venation (Lamina)	+	-	-	-	-	-	-	-
6	Ground Tissue parenchyma (Midrib)	+	+	-	-	-	-	+	-
7	Bundle sheath	+	+	+	+	-	-	-	-
8	Vascular bundle	+	-	-	-	-	-	-	-
9	Lower Epidermis	+	-	-	-	-	-	-	-

A. Saffranin; B - Fast Green; C- Coomasie Blue; D- I₂KI ;

E - Sudan IV; F - Dragendroff; G - Ferric chloride; H - Vannilin

Table 3. Attempt towards localization of specific phytochemicals in different cell types of the petiole in *Kigelia pinnata* DC

S.No	Tissue Type	A	B	C	D	E	F	G	H
1	Epidermis	+	-	-	-	-	-	-	-
2	Sub Epidermis	+	-	+	+	-	-	-	-
3	Outer cortex	+	-	-	-	-	+	+	+
4	Inner cortex	+	-	-	-	-	-	-	-
5	Endodermis	+	+	+	+	-	-	-	-
6	Xylem	+	-	-	-	-	-	-	-
7	Phloem	+	+	+	+	-	-	+	+
8	Parenchymatous pith	+	+	+	-	-	-	+	-

A- Saffranin ; B - Fast Green; C- Coomasie Blue; D- I₂KI ;

E- SudanIV; F- Dragendroff; G- Ferric chloride; H - Vannilin