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Preliminary Screening of Phytochemicals from Methanolic Extract of *Kigelia Pinnata* Flowers

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ABSTRACT

Kigelia pinnata is a medium to large sized, upto 25 m in height, evergreen tree with a spreading crown belonging to the family Bignoniaceae. It is commonly known as African sausage tree/ Cucumber tree. It is one of the most important species in avenue plantations. The whole plant is also medicinally important as it contains enormous number of phyto-constituents which helps in curing ailments. The GC - MS analysis results of Kigelia pinnata flowers showed a total of 25 compounds, of which 4-vinylphenol, sucrose, aspartame, 21, 25-dihydroxy cholecalciferol, 2-fluorobuta-1,3-diene and 2-methoxy 4-vinylphenol showed greatest contribution in percentage to the total area. The screened phytochemicals are known to possess anti-inflammatory, anti-microbial and anticancerous properties.
Keywords: *Kigelia pinnata, flowers, methanol extraction, GC-MS, phytochemicals, anti-microbial and anti-inflammatory*

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INTRODUCTION

Natural plant based phytochemicals are known to contain active principle compounds that can be used for therapeutic purposes. In plants, phytochemicals act as a natural defence system for host plants and provide colour, aroma and flavour to them. Plant phytochemicals play a role in the interaction of the cell (organism) with its surroundings, ensuring its continued existence in the environment [1]. *Kigelia pinnata* is a medium to large sized, upto 25 m in height, evergreen tree with a spreading crown belonging to the family Bignoniaceae. It is commonly known as African sausage tree/ Cucumber tree. The bark is generally grey with leathery leaves, roughly hairy on both surfaces. Flowers are striking, dark maroon to red with unpleasant smell [2]. With its fast growth rate, spreading canopy and interesting flowers, it makes a good avenue tree and the tree is also popular for its various phytochemicals from its leaves, bark, fruits and roots which are known to have many medicinal properties, whereas scanty literatures are available regarding the chemical constituents of its flowers and hence the present investigation is undertaken. The main objective of the present study is to identify the chemical constituents present in the flowers of *Kigelia pinnata*.

MATERIAL AND METHODS

FLOWER COLLECTION

Fresh, dark maroon flowers of *Kigelia pinnata* were collected from the tree in Forest College and Research Institute, Mettupalayam, Tamil Nadu and shade dried at room temperature with constant turning to inhibit fungal growth. The dried flowers were later crushed to obtain a coarse powder for easy extraction using soxhlet apparatus.

PREPARATION OF METHANOL EXTRACT

Exactly 5.0 grams of the crushed maroon flowers of *Kigelia pinnata* were extracted with 25 mL of methanol in an automated soxhlet apparatus (SOXTEC 2043 FOSS). The extraction was performed at 60^0 C for 2 hours and 30 minutes completing three cycles. All the phytoconstituents were extracted from the flowers at the end of the third cycle. The extract was then dried at room temperature and stored at 4^0C in air tight sterile vials in the refrigerator.

GC - MS ANALYSIS

The chemical composition of the methanolic flower extract was analysed using Thermo GC - Trace Ultra Ver: 5.0 and Thermo MS DSQ II fitted with a DB 35 - MS capillary standard non - polar column (30 m, ID: 0.25 mm and film thickness of 0.25 μm). 0.5 μl of methanol extract was injected for analysis and Helium was used as a carrier gas at 1 mL/ min. The instrument was set as follows, Injector port temperature set to 250^0 C, source kept at 220^0 C. The oven temperature was programmed from 70^0 C to 260^0 C at the 6^0 C/ min rate. The MS was set to scan from 50 - 650 Da. The MS also had inbuilt pre - filter which reduced the neutral particles. The data system has two inbuilt libraries for searching and matching the spectrum, NIST4 and WILEY9 containing more than five million references.

IDENTIFICATION OF COMPOUNDS

Interpretation of mass spectrum of GC - MS was done using the database of National Institute Standard and Technology (NIST4) and WILEY9 [3]. The spectrum of the unknown component was compared with the spectrum of the known components stored in the inbuilt library.

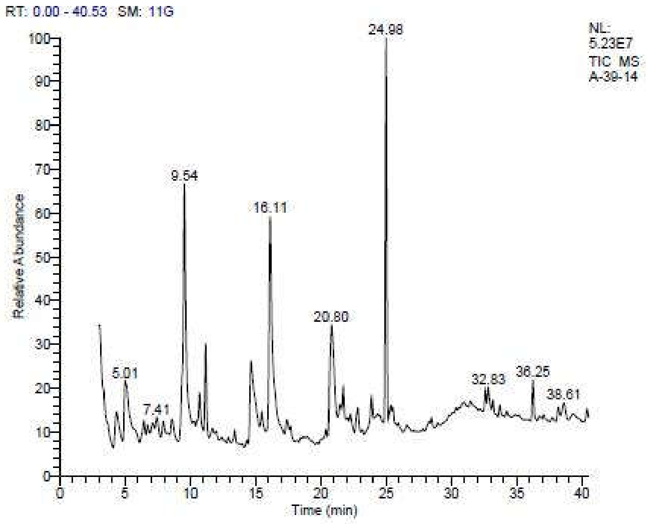
RESULTS

The GC - MS analysis results of *Kigelia pinnata* flowers are summarised in Table 1, of which 4-vinylphenol, sucrose, aspartame, 21, 25-dihydroxy cholecalciferol, 2-fluorobuta-1,3-diene and 2-methoxy 4-vinylphenol showed the greatest contribution in percentage to the total area. Also, the compounds like Glucobrassicin, 7-Quinolinol, Hexadecene, Ethyl α-hydroxy O-nitrocinnamate, -3-(2-Methoxyphenyl)-2-propenoic acid, n-Tetracosanol-1, 08217205002 Flavonol and Gibberellin A8 methyl ester were found to be present at lower concentrations. The chromatograph of methanolic extract from *Kigelia pinnata* flowers by GC - MS is given in Figure 1.

Table 1. Phytochemicals identified in *Kigelia pinnata* flower extract

S.No.	RT (min)	Compound name	Molecular formula	Molecular weight	Area (%)
1.	4.32	4-Methylthiobut-1-ene	$C_5H_{10}S$	102	2.90
2.	4.99	2-Fluorobuta-1,3-diene	C_4H_5F	72	5.47
3.	6.45	Dimethyl ester of tartronic acid	$C_5H_8O_5$	148	1.52
4.	7.41	6-Methoxy-3(2H)-Pyridazinone	$C_{13}H_{12}N_2O_4$	260	1.27
5.	7.92	Glucobrassicin	$C_{16}H_{19}N_2O_9S_2$	447	0.96
6.	8.58	Debrisoquine	$C_{10}H_{13}N_3$	175	1.20
7.	9.54	4-Vinylphenol	C_8H_8O	120	14.25
8.	10.68	5-Hydroxymethyl furfural	$C_6H_6O_3$	126	2.00
9.	11.16	2-Methoxy-4 vinylphenol	$C_9H_{10}O_2$	150	3.64
10.	13.41	Hexadecene	$C_{16}H_{32}$	224	0.70
11.	14.63	21,25-Dihydroxycholecalciferol	$C_{27}H_{44}O_3$	416	6.63
12.	15.47	7-Quinolinol	C_9H_7NO	145	0.79
13.	16.11	Sucrose	$C_{12}H_{22}O_{11}$	342	12.74
14.	18.92	Ethyl α-hydroxy O-nitrocinnamate	$C_{11}H_{11}NO_5$	237	0.69
15.	20.80	Aspartame	$C_{14}H_{18}N_2O_5$	294	9.53
16.	21.71	Palmitic acid, methyl ester	$C_{17}H_{34}O_2$	270	2.01
17.	22.26	-3-(2-Methoxyphenyl)-2-propenoic acid	$C_{10}H_{10}O_3$	178	0.89
18.	23.89	Furoscrobiculin B	$C_{15}H_{20}O_2$	232	1.71
19.	24.98	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	16.48
20.	28.45	n-Tetracosanol-1	$C_{24}H_{50}O$	354	0.94
21.	30.35	08217205002 Flavonol	$C_{26}H_{30}O_{16}$	598	0.98
22.	32.83	Glycerol 1-palmitate	$C_{19}H_{38}O_4$	330	2.20
23.	36.25	Celidoniol Deoxy	$C_{29}H_{60}$	408	1.78
24.	38.61	1,5,6-Trihydroxy-2-methylanthraquinone	$C_{24}H_{34}O_5Si_3$	486	1.48
25.	39.36	Gibberellin A8 methyl ester	$C_{20}H_{26}O_7$	378	0.97

Figure 1. Chromatograph of methanolic extract of *Kigelia pinnata* flowers by GC-MS



DISCUSSION

The rise in demand to study the plant kingdom, which is one of the richest sources of promising versatile chemical compounds, is growing persistently throughout the world during the last few decades. Plants could play a great role in exploring new curative resources against the threat of new and recent diseases.

The current investigation on chemical composition from the flowers of *Kigelia pinnata*, the compound dibutyl phthalate is considered to be a contaminant in the methanol extract as the solvent leaches out the plastic material from the vials in which the extract was stored [4]. Hence the compound should be neglected from the chemical components screened. The compound 2-Methoxy-4 vinylphenol was found to present with the percentage of 3.64. The pharmaceutical uses of 2-Methoxy-4 vinylphenol analysed from *Mussaenda frondosa* were reported [5]. This phenolic group has antimicrobial, antioxidant, anti inflammatory and analgesic properties. The compound is also reported to be responsible for inhibiting germination in wheat seeds [6].

The phytochemical 24, 25 – dihydroxycholecalciferol also known as 24, 25 – dihrodroxy vitamin D_3 with 6.63% is reported to be found mainly in the Solanaceae family. With research focussed on its leaves, the compound is known to be poisonous when produced in large amounts [7]. Basic knowledge about the biosynthesis of vitamin D_3 in photosynthetic organisms is still lacking and any breakthrough in the synthesis process will help us to manipulate the content to produce plants with a higher natural amount of vitamin D_3. The presence of sucrose (12 %) in *Kigelia pinnata* flowers is predominantly to attract pollinators (bats) and possibly influence pollinator's movement in a way that benefits the plants. Studies showed that some species of fruit eating and nectar feeding bats prefer sucrose over other hexose [8].

The phytochemical aspartame was found in flowers of *Kigelia pinnata* with an area percentage of 9.53. Aspartame is primarily derived from compounds called amino acids. These are chemicals which are used by plants and animals to create proteins that are essential for life. Of the 20 naturally occurring amino acids, two of them, aspartic acid and phenylalanine, makes the compound aspartame. Aspartic acid is one of five amino acids that have a "charged" side group. The charged side group on aspartic acid is ($-CH_2-COOH$). When put in water, this material ionizes and becomes negatively charged whereas, phenylalanine has a nonpolar, hydrophobic side group which is not compatible with water. It is made up of a six carbon ring and is attached to the main amino acid backbone via a methyl ($-CH_2$) group. Prior to synthesis into aspartame, it is reacted with methanol and is converted to a methyl ester [9]. In the current investigation, the use of methanol as a solvent for extraction of phytochemicals might have reacted with the amino acids present in the flower henceforth producing the compound aspartame.

The compound glucobrassicin was found to be present in minimum percentage (0.96 %). Glucobrassicin is a type of glucosinolate that can be found in almost all cruciferous plants. The presence of glucosinolates in plants are well known for their defence capacity against herbivores and microbial pathogens. Thereby, glucosinolates can also contribute to control microbial infectious disease in humans and some special breakdown products of glucosinolate even support cancer prevention. Glucosinolates as phytoanticipins are constitutively present in the plant, but after a herbivore or pathogen attack, different types of glucosinolates are induced as a defence mechanism. These different chemical compounds are important for the resistance against the different aggressors [10]. Hence, the flowers of *Kigelia pinnata* can be used as a viable option to extract compounds which are of remunerative value.

CONCLUSION

Kigelia pinnata, an ornamental tree whose presence in avenue plantation is inevitable because of its attractive flowers have much more to offer because it is rich in many secondary metabolites which can be used for the manufacture of many novel products whose utility expands from pharmacognostical value, industrial application and also has a role in agricultural sector.

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