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PRELIMINARY PHYTOCHEMICAL SCREENING AND GC MS ANALYSIS OF CHLOROFORM EXTRACT OF *KANDELIA CANDEL* (L.) DRUCE

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ABSTRACT: The aim of the present study is to screen the phytochemicals present in the leaf extract of *Kandelia candel* and further analysis of the bioactive components present in it by Gas chromatography - Mass spectrometry (GC-MS) analysis. Carbohydrates, proteins, amino acids, Saponins, flavonoids, terpenoids, glycosides, tannins, alkaloids, and phenols were present in the sample. The percentage yield of plant extract is high in ethanol extract and is 7.02%. GC-MS analysis of chloroform extract of *K.candel* leaf extract revealed the presence of 16 compounds and Longipinene epoxide(peak area 25.12) and 2-$C_{14}H_{26}$ (peak area 25.12)were the major compounds. The compounds were identified by comparing their retention time, peak area and by interpretation of mass spectra and matched with the National Institute of Standards and Technology (NIST) library.

INTRODUCTION: Medicinal plants importance to the health of people depend on plant based traditional medicine for their primary health care. Plant derived medicines are relatively safer than synthetic alternatives. World Health Organization supports the use of traditional medicine provided they are proven to be efficacious and safe. Medicinal plants have been used for centuries as remedies for human ailments and diseases because they contain components of therapeutic values. Also they are less toxic to humans and environmentally friendly due to less pollutant produced in production and have minimal health hazards.

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The specific region were these plants occur are termed as mangrove ecosystem. Mangroves are usually found only in tropical climates as they need consistently warm condition for development and survival and South India is the best place in India for the establishment of the mangroves. Mangroves form a special type of Eco tone, which is significant in the conservation and protection of wetlands. Mangroves represent a highly dynamic and fragile ecosystem.

Coastal belt of Kerala boasted on about700 km^2 of mangrove forest, which has now dwindled to 17 km^2, with Kannur district claiming the largest area of mangroves in the state, estimated at 755 ha[1]. Total of 15 pure mangroves species were recorded from the mangrove areas of Kerala and are belonging to 9 genera and 6 families. Rhizophoraceae represented 7 species followed by two each from families, Avicenniaceae, Sonnaraceae and Euphorbiaceae respectively [2].

In Kannur mangroves are scattered and distributed in the area of Pappinissery, Kunhimangalam, Tellicherry, Edakkad *etc*. Tellichery is characterized by Avicennia-Sonneratia-Rhizophora combination. Pappinissery has Kandelia - Avicennia - Aegiceras group. Kunhimangalam shows Excoecaria – Avicennia – Rhizophora - Bruguiera belt [3].

Studies on mangrove plant parts and its major chemical classes displayed various level of biological activities such as antibacterial, antifungal, antiplasmodial, cytotoxic, hepatoprotective, and free radical scavenging activities [4, 5] and [6].

Extract of mangrove plants are rich source of steroids, diterpenes and triterpenes, saponins, flavonoids, alkaloids and tannins. [7] Mangrove plants are a rich source of steroids, triterpenes, saponins, flavonoids, alkaloids and tannins [8] and [9, 10]. *Kandelia candel* is a species of mangrove in the family Rhizophoraceae. Occurs locally on banks of tidal rivers among other mangrove species. Evergreen shrub or small tree, up to 7M tall. Bark of *Kandeliacandel* is used for diabetes.

MATERIALS AND METHODS:

Plant Collection: *Kandelia candel* (L) Druce collected from Valapattanam, Kannur district, Kerala was taken for the study. The collected plants were washed thoroughly with tap water followed with distilled water for the removal of dust and soil particles. The plants cut into pieces were shade dried at room temperature for 15 days then coarsely powdered and used for extraction.

Preparation of Extract: The powder (40 gm.) was extracted with chloroform, ethyl acetate and ethanol in a soxhlet apparatus (3840; Borosil Glass works Ltd., Mumbai, India) in increasing order of their polarity. Finally the dried powder was macerated using water with constant stirring for 48 hours using the orbital shaker (Rivotek; Riviera Glass Pvt. Ltd., Mumbai, India) and the extract was filtered. The extracts were concentrated, dried and stored at -20 ºC in the deep freezer (RQV- 300; plus, REMI electro technik Ltd., Thane, Maharashtra, India) for further analysis.

Extract Recovery Percent: The amount of extract recovered after successive extraction was weighed and the percentage yield was calculated by the following formula,

$$\text{Extract Recovery Percent} = \frac{\text{Amount of extract (g)}}{\text{Amount of plant sample (g)}} \times 100$$

Qualitative Preliminary Phytochemical Analysis: For preliminary phytochemical screening, plant extract was subjected to various qualitative chemical tests to determine the presence of various phyto-constituents like glycosides, tannins, phytosterols, proteins, amino acids, carbohydrates, flavonoids, phenolic compounds, according to standard methods recommended by Raaman, 2006 [11].

GC –MS Analysis: GC-MS analysis of chloroform extract of leaf of *Kandelia candel* were performed using Themo Scientific Trace 1300 Gas chromatograph equipped with ISQ- QD Mass spectrometer with TG-5MS column (30mm × 0.25mm ID ×0.25µm). Helium gas (99.999%) was used as the carrier gas at constant flow rate 1ml/minute and an injection volume of 1µl was employed. An injection port temperature of 280 ºC and an ion-source temperature of 200 ºC were set. The oven temperature was programmed from 60 ºC for 3 minutes with an increase of 5 ºC /minute to 240 ºC with a hold time of 3 minutes. Then temperature was increased at a rate of 35 ºC/min till 280 ºC with a hold time of 5 minutes. Scan interval was programmed for 0.2 seconds with a mass range of 40 - 450 amu. Total GC running time was 45 minutes. The components in the extract were identified based on the mass spectra of NIST library data.

RESULT AND DISCUSSION: The highest yield of extract of *Kandelia candel* leave was found with ethanol and the lowest with ethyl acetate and the details are provided in **Table 1**. Phytochemical screening of the sequential extracts of *K. candel* leaves revealed the presence of various bioactive components of carbohydrate, protein, amino Acids, alkaloids, flavonoids, tannin, triterpenoids, phenols, glycosides, cardiac glycosides, as presented in **Table 2**.

GC-MS chromatogram of chloroform extract of the plant leaves revealed 16 peaks indicating sixteen phytochemical constituents. The major compounds identified with their retention time, molecular formula, molecular weight and peak area are presented in **Fig. 1**, **Table 3**. Among them Longipinene epoxide (peak area 25.12) and 2-$C_{14}H_{26}$ (peak area 25.12) were major compounds.

TABLE 1: EXTRACTIVE YIELD OF PLANT EXTRACTS OF *K.CANDEL* LEAF IN DIFFERENT SOLVENTS

Sl no.	Solvent	Yield (%)
1	Chloroform	4.52
2	Ethyl acetate	0.24
3	Ethanol	7.02

TABLE 2: PHYTOCHEMICAL SCREENING TESTS FOR VARIOUS PHYTOCHEMICALS OF *K. CANDEL* LEAF

Sl no.	Phytochemicals	Chloroform	Ethyl Acetate	Ethanol
1	Carbohydrate	+	+	+
2	Protein	-	-	+
3	Amino acid	-	-	+
4	Alkaloid	+	+	-
5	Flavonoid	+	-	+
6	Tannin	+	-	-
7	Terpenoid	+	-	+
8	Saponin			
9	Phenol	+	-	+
10	Glycosides	-	+	-
11	Cardiac Glycosides	-	-	-

+ indicates presence of phytochemicals and – indicates absence of phytochemicals

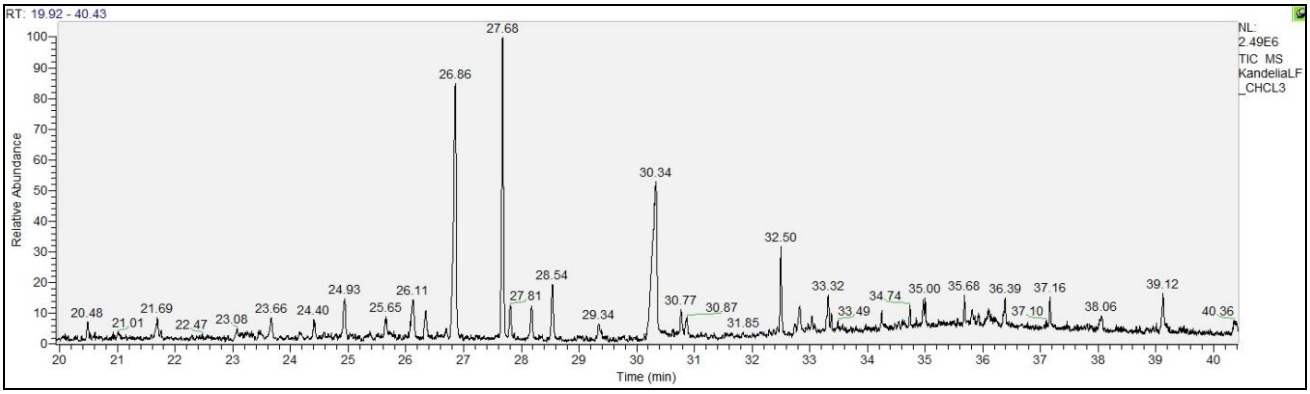

FIG. 1: GC- MS CHROMATOGRAM OF CHLOROFORM EXTRACT OF *K.CANDEL* LEAF

TABLE 3: GC MS ANALYSIS OF *K.CANDEL* LEAF CHLOROFORM EXTRACT

Sl no	Rt	Compound Name	Peak Area	Molecular Formula	Molecular Weight
1	20.48	phenol, 4-(1,1-dimethylethyl)-2-(1,1-dimethylpropyl)-	0.42	$C_{15}H_{24}O$	220
2	21.69	(2S,3S)-(-)-3-Propyloxiranemethanol	0.84	$C_6H_{12}O_2$	116
3	23.66	2-Cyclohexen-1-one, 4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-, [R-[R*,R*-(E)]]-	0.73	$C_{13}H_{20}O_2$	208
4	24.40	2,6-Dimethyl-3,5,7-octatriene-2-ol, ,E,E-	0.47	$C_{10}H_{16}O$	152
5	24.93	Bicyclo[5.1.0]octane, 8-(1-methylethylidene)-	2.28	$C_{11}H_{18}$	150
6	25.65	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	0.80	$C_{10}H_{12}O_3$	180
7	26.11	4-Methyloctanoic acid	2.62	$C_9H_{18}O_2$	158
8	26.86	Longipinene epoxide	25.12	$C_{15}H_{24}O$	220
9	27.68	2-$C_{14}H_{26}$	25.12	$C_{14}H_{26}$	194
10	27.81	Pentanal, 2,4-dimethyl-	1.61	$C_7H_{14}O$	114
11	28.54	1-Octadecyne	1.52	$C_{18}H_{34}$	250
12	30.34	n-Hexadecanoic acid	21.29	$C_{16}H_{32}O_2$	256
13	30.77	Hydroperoxide, heptyl	1.02	$C_7H_{16}O_2$	132
14	32.50	Propanoic acid, 2-methyl-, 2-ethyl-1-propyl-1,3 -propanediyl ester	3.89	$C_{16}H_{30}O_4$	286
15	33.32	Oxirane, [(tetradecyloxy)methyl]-	1.79	$C_{17}H_{34}O_2$	270
16	35.00	Oxalic acid, allylnonyl ester	0.66	$C_{14}H_{24}O_4$	256

CONCLUSION: The results evidently specifies that chloroform leaf extract of *K. candel* contains various bioactive compounds have various medicinal properties.

However, isolation of individual phytochemical constituents and subjecting it to the biological activity will definitely give fruitful results. Therefore, it is recommended as a plant of phytopharmaceutical importance.

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CONFLICT OF INTEREST: The authors declare no known conflict of interest.

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