

**GC-MS ANALYSIS OF BIOACTIVE COMPONENTS OF *KANDELIA CANDEL* (L.) DRUCE****Jasna T K *, K M Khaleel**

Department of P G Studies and Research in Botany, Sir Syed College, Taliparamba, Kannur, Kerala, India

*Corresponding author: jasnathaikandy@gmail.com**ABSTRACT**

Kandelia candel a mangrove belonging to the family Rhizophoraceae, a plant traditionally used to cure diabetics. The present study was carried out to screen the phytochemicals present in the bark extract of *Kandelia candel* and GC-MS evaluation of chemical constituents from chloroform extract. The plant parts were sequentially extracted in different solvents like petroleum ether, chloroform, acetonitrile and water. The percentage yield of plant extract is high in acetonitrile extract. 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. Carbohydrates, proteins, amino acids, flavonoids, saponin, tannins, alkaloids, and phenols were present in the sample. GC-MS analysis of chloroform extract of *K. candel* bark extract revealed the presence of 24 compounds and Phenol, 2,5-bis (1,1-dimethylethyl); 4''a-Methyl-8''-methylidene decahydro-2''H-dispiro[bis(cyclopropane)-1,1':2'], and Docosahexaenoic acid, 1,2,3-propanetriyl ester were the major compounds. The compounds identified through the GC-MS analysis have various bioactivities. 1- Heptatriacotanol and digitoxin are few among them.

Keywords: Bioactive compounds, Chloroform extract, Digitoxin, GC MS, *Kandelia candel***1. INTRODUCTION**

Vegetables, spices and fruits contain phytochemicals have main role against diseases. They also act as radical scavenger by inhibiting oxidation process through several mechanisms. Compounds with bioactivities present in medicinal plant are analyzed by using these techniques. Traditional practices used medicinal plants and it act as the backbone of the traditional medicine. Medicinal values of the substance present in the plants are yet to be discovered and the screening of active compounds has become very significant [1]. In traditional medicine the medicinal plants act as back bone and are the potential source of bioactive compounds for the drug development [2]. About 80% of the population in developing countries depends up on traditional medicine for their healthcare needs.

Usage of various parts of medicinal herbs is recommended by the traditional medicine system in the form of powder or extracts. Medicinal plants are used as natural remedies and they are found to be effective and safe and are used to treat various ailments. Natural products derived from plants are used as food, clothing, tools, medicine etc. For the discovery of new drugs and drug development, medicinal plant acts as important source. Secondary metabolites are developed as new

drugs and they are useful for mankind [3]. According to WHO, plant product are used as alternative or complementary medicine in 80% of the developing countries [4]. Secondary metabolites with numerous biological activities are present in plants [5]. Screening of medicinal plants to check the presence of bioactive compound is necessary [6].

GC MS technique has been used successfully to identify the structures of various phytochemicals in biological sample and plant extracts. For the identification and quantification of phytochemical, GC MS is used as common technique. Complex mixture with an unknown organic compound can be determined by matching the spectra with reference spectra [7]. Traditionally, for the treatment of several ailments, plants are used as medicine and they are free from side effects. The aim of the study was to analyze the acetonitrile extract of bark to elucidate the chemical composition by GC MS analysis.

2. MATERIAL AND METHODS**2.1. 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.

2.2. Preparation of Extract

The powder (40 gm) was extracted with petroleum ether, chloroform and acetonitrile 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°C in the deep freezer (RQV- 300; plus, REMI electro technik Ltd., Thane, Maharashtra, India) for further analysis.

2.3. Extract Recovery Percent

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

Extract Recovery Percent = [Amount of extract (g)/Amount of plant sample (g)] x 100

2.4. Qualitative Preliminary Phytochemical Analysis

For preliminary phytochemical screening, plant extract was subjected to various qualitative chemical tests to determine the presence of various phytoconstituents like glycosides, tannins, phytosterols, proteins, amino acids, carbohydrates, flavonoids, phenolic compounds, according to standard methods recommended by Raaman [8].

2.5. GC-MS Analysis

GC-MS analysis of chloroform extract of bark of *Kandelia candel* were performed using Thermo 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 of 1ml/minute and an injection volume of 1µl was employed. An injection port temperature of 280°C and an ion-source temperature of 200°C were set. The oven temperature was programmed from 60°C for 3 minutes with an increase of 5°C /minute to 240°C with a hold time of 3 minutes. Then temperature was increased at a rate of 35°C /min till 280°C 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.

3. RESULTS AND DISCUSSION

3.1. Extract yield percentage

In phytochemical analysis the initial step is the extraction, to separate the compound from the plant powder. The extraction by different solvent is used for the best separation of compounds. Gradual isolation of the groups was done by using solvent with different polarity. Yield Percentage of the extracts obtained by using various solvents such as petroleum ether, chloroform, acetonitrile and water were given in Table 1. The extraction yield was found minimum for petroleum ether and maximum for acetonitrile.

The highest yield of extract of *Kandelia candel* bark was found with acetonitrile and the lowest with petroleum ether and the details are provided in Table 1.

Table 1: Extract yield percentage in different solvents

Sl No	Solvent	Yield (%)
1	Petroleum ether	1.98
2	Chloroform	4.76
3	Acetonitrile	11.75
4	Aqueous	10.86

3.2. Phytochemical screening

The different parts of the plant and their extracts were studied for the presence of several classes of phytochemicals by performing appropriate tests. Preliminary phytochemical analysis of different extracts of *K. Candel* bark inferred as it is a good source of phytochemicals. The results observed are shown in Table 2. The results of phytochemical screening shows the presence of carbohydrate, protein, amino acid, alkaloid, saponins, phenolics, tannins, flavonoids, glycosides and terpenoids in most of the tested extracts.

Phytochemical screening of the sequential extracts of *K. candel* bark revealed the presence of various bioactive components like carbohydrate, protein, amino Acids, alkaloids, flavonoids, tannin, triterpenoids, phenols, glycosides, cardiac glycosides, as presented in Table 2. Phytochemicals are plant chemicals and non-nutritive which have properties like protection and prevention [9]. Different phytochemical present in the plant have wide range of activities. Flavonoid and tannin found to have anti-diarrheal activity [10]. Flavonoids are the

phytochemical which possess various activity like anti-inflammatory, antimicrobials and anticancer activity [11].

Table 2: Preliminary Phytochemical Analysis of *K.candel* bark

	Chloroform	Acetoni-trile	Water
Carbohydrate	+	+	+
Protein	-	-	+
Amino acid	-	-	+
Alkaloid	+	+	+
Saponins	-	+	+
Phenolics	+	+	+
Tannins	+	+	+
Flavonoids	+	+	+
Glycosides	-	+	-
Cardiac glycosides	-	-	-
Terpenoids	-	+	+

Positive (+) present, Negative (-) absent

3.3. GC MS Analysis

3.3.1. GC MS analysis of *Kandelia candel* bark chloroform extract

GC MS analysis of *K. candel* chloroform extract of bark shows presence of 24 compounds, majority of the compound identified in bark extract have bioactivities. The compounds identified with their retention time, molecular formula, molecular weight and peak area are presented in Fig. 1, Table 3. Phenol, 2,5-bis (1,1-

dimethylethyl); 4''a-Methyl-8''-methylidene decahydro-2''H-dispiro [bis(cyclopropane)-1,1':2', and Docosahexaenoic acid, 1,2,3-propanetriyl ester were the major compounds. 2-Piperidinone, N-[4-bromo-n-butyl]; 3-Trifluoroacetoxy-pentadecane; Cholest-22-ene-21-ol, 3, 5-dehydro-6-methoxy-, pivalate; Digitoxin; 1-Octanol, 2-butyl-; Dodecane, 1, 2-dibromo; Z, Z, Z-1, 4, 6, 9-Nonadecatetraene; heptadecane, 9-hexyl-; 4-Trifluoroacetoxytridecane; 1- Heptatriacotanol; Tert-Hexadecanethiol; Cholesta-8, 2 4-dien-3-ol, 4-methyl-, (3á, 4à) -and 2-[4-methyl-6-(2, 6, 6-trimethylcyclohex-1-enyl) hexa-1, 3, 5-trienyl] cyclohex-1-en-1-carboxaldehyde are the bioactive compounds present in the bark extract.

2-Piperidinone, N-[4-bromo-n-butyl] is alkaloid in nature and has antimicrobial, antioxidant, anti-inflammatory activity [12]. 3-Trifluoroacetoxy-pentadecane has bioactivities such as anti-nephrotoxic and antioxidant activities [13]. Cholest-22-ene-21-ol, 3, 5-dehydro-6-methoxy-, pivalate is a steroid compound with antimicrobial, anti-inflammatory, antiarthritic, antiuretic and antiasthmatic activities [14]. Digitoxin is an anticancer agent and cardiac glycoside [15]. 1-Hexadecanol, 2-methyl- is alcoholic in nature with antimicrobial activities, 1-Octanol, 2-butyl-shows antimicrobial activity [16]. Dodecane, 1, 2-dibromo-antibacterial, Z, Z, Z-1, 4, 6, 9-Nonadecatetraene have antibacterial activity. In vitro antifungal potentials of bioactive compound heptadecane, 9-hexyl was reported by Abubacker and Kamala Devi [17].

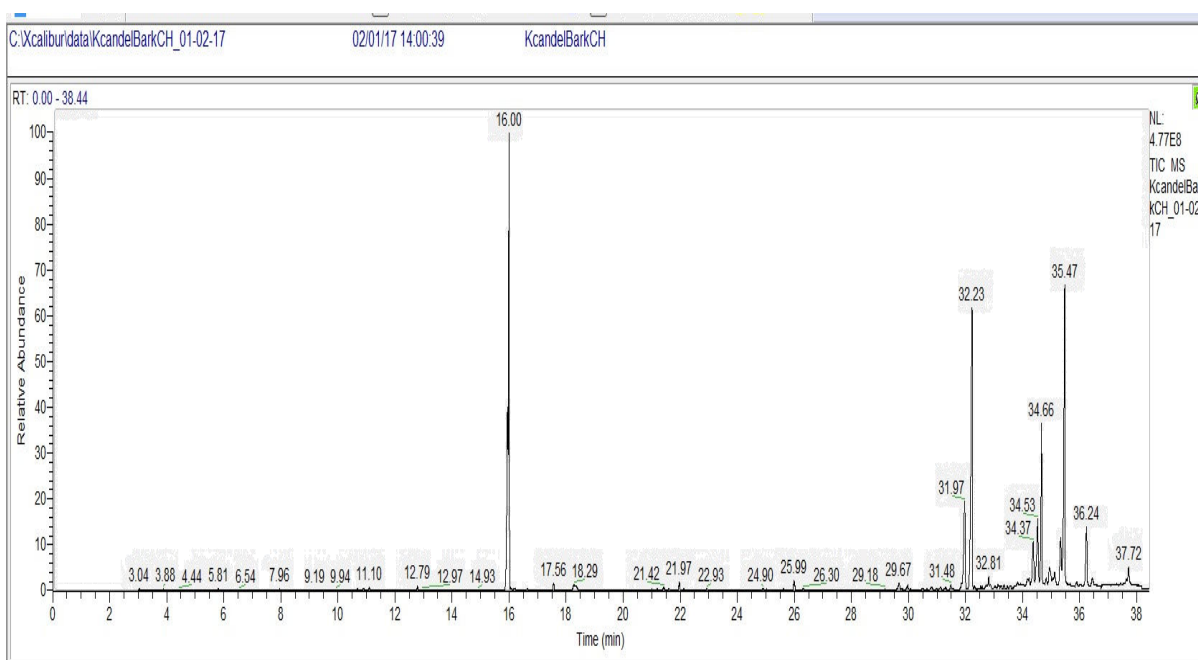


Fig. 1: GC MS analysis of *Kandelia candel* bark chloroform extract

Table 3: GC MS analysis of *K. candel* bark chloroform extract

RT	Compound name	Peak area	Molecular formula	Molecular weight
16	Phenol, 2,5-bis(1,1-dimethylethyl)-	24.21	C ₁₄ H ₂₂ O	206
21.97	cis-2-Methyl-7-octadecene	0.38	C ₁₉ H ₃₈	266
25.99	Hexadecen-1-ol, trans-9-	0.51	C ₁₆ H ₃₂ O	240
29.67	cis-2-Methyl-7-octadecene	0.41	C ₁₉ H ₃₈	266
32.23	4''a-Methyl-8''-methylidene decahydro-2''H-dispiro[bis(cyclopropane)-1,1':2',	18.58	C ₁₈ H ₂₄ O ₂	272
35.47	Docosahexaenoic acid, 1,2,3-propanetriyl ester	15.32	C ₆₉ H ₉₈ O ₆	1022
36.24	cis-5,8,11,14,17-Eicosapentaenoic acid	3.59	C ₂₀ H ₃₀ O ₂	302
21.19	2-Piperidinone, N-[4-bromo-n-butyl]-	0.05	C ₉ H ₁₆ BrNO	233
37.72	3-Trifluoroacetylpentadecane	0.99	C ₁₇ H ₃₁ F ₃ O ₂	324
34.18	Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate	0.43	C ₃₃ H ₅₄ O ₃	498
33.25	Digitoxin	0.17	C ₄₁ H ₆₄ O ₁₃	764
35.66	1-Hexadecanol, 2-methyl-	0.07	C ₁₇ H ₃₆	256
25.87	1-Octanol, 2-butyl	0.02	C ₁₂ H ₂₆ O	186
28.27	Dodecane, 1,2-dibromo-	0.01	C ₁₂ H ₂₄ Br ₂	326
29.18	Z,Z,Z-1,4,6,9-Nonadecatetraene	0.02	C ₁₉ H ₃₂	260
29.42	Heptadecane, 9-hexyl	0.01	C ₂₃ H ₄₈	324
30.27	4-Trifluoroacetyltridecane	0.01	C ₁₅ H ₂₇ F ₃ O ₂	296
30.51	1-Heptatriacotanol	0.07	C ₃₇ H ₇₆ O	536
33.04	tert-Hexadecanethiol	0.25	C ₁₆ H ₃₄ S	258
33.13	Cholesta-8,24-dien-3-ol, 4-methyl-, (3á,4à)-	0.34	C ₂₈ H ₄₆ O	398
33.57	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	0.16	C ₂₃ H ₃₂ O	324
33.93	Cholesta-5,7,9(11)-trien-3-ol acetate	0.29	C ₂₉ H ₄₄ O ₂	424
34.82	9-Octadecen-12-ynoic acid, methyl ester	0.26	C ₁₉ H ₃₂ O ₂	292.

4-Trifluoroacetyltridecane [18] and 1- Heptatriacotanol have antimicrobial activity [19]. Tert-Hexadecanethiol possesses antioxidant and antibacterial activity [20]. Cholesta-8, 2 4-dien-3-ol, 4-methyl-, (3á, 4à) - steroid compound have antimicrobial, diuretic, anti-inflammatory and antiasthmatic activity [16]. 2-[4-methyl-6-(2, 6, 6-trimethylcyclohex-1-enyl) hexa-1, 3, 5-trienyl] cyclohex-1-en-1-carboxaldehyde have antimicrobial and anti-inflammatory activities [16].

4. CONCLUSION

Medicinal plants are enriched with bioactive compounds and efforts are being made for the isolation of more potent compounds. GC MS analysis showed the presence of 24 compounds. Bioactive compounds derived from these plant acts as a source of antioxidant, antimicrobial and anti-inflammatory properties. Compounds present in the plant justify the use of the plant for various diseases. Isolation of individual phytochemical constituents and the pharmacological analysis will give fruitful results.

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Conflict of interest

The authors declare no known conflict of interest.

6. REFERENCES

1. Arokiyaraj S, Radha R, Martin S, Perinbam K. *Indian Journal of Science and Technology*, 2008; **1**:1-4.
2. Khalaf NA, Shakya AK, Al-othman A, Ahbar Z, Farah H. *Turkish Journal of Biology*, 2007; **31**:1-5.
3. Velayutham P, Karthi C. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2015; **7**:260-267.

4. Henok GA, Sahabjada, Asif J, Arshad MD, Tabarak M. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2017; **9**:29-34.
5. De-Fatima A, Modolo LV, Conegero LS, Pilli RA, Ferreira CV, Kohn LK, et al. *Current Medicinal Chemistry*, 2006; **13**:3371-3384.
6. Saha MR, Alam MA, Akter R, Jahangir R. *Bangladesh Journal of Pharmacology*, 2008; **3**:90-96.
7. Anjali R, Rasika T, Amruta T, Vedavati P, Nirmala D. *International journal of chemtech research*, 2009; **1**:158-161.
8. Raaman N. *Phytochemical techniques*. New Delhi, New India Publishing Agency, Jai Bharat Printing Press. 2006; 19-22.
9. Kumar A, Ilavarasan R, Jayachandran T, Decaraman M, Aravindhnan P. *Pakistan Journal of Nutrition*, 2009; **8**:83-85.
10. Enzo AP. Traditional plants and herbal remedies used in the treatment of diarrheal disease: Mode of action, quality, efficacy and safety considerations. In: Ahmad I, Aqil F, Qwais M, Modern Phytomedicine Turning Medicinal Plants in to Drugs. WILEY-VCH VerlagGmbH & Co. KGaA, Weinheim, 2007; 248-260.
11. Yamato G. *Journal of clinical investigation*, 2002; 493-503.
12. Meenakshi VK, Gomathy S, Senthamarai S, Paripooranaselvi M, Chamundeswari KP. *Journal of Current Chemical and Pharmaceutical Science*, 2012; **2(4)**:271-276.
13. Haider MH, Imad HH, Omar AI. *International Journal of Pharmacognosy and Phytochemical Research*, 2016; **8(3)**:369-385.
14. Sunita A, Ganesh K, Sonam M. *Asian Journal of Pharmaceutical and Clinical Research*, 2017; **10(9)**: 64-69.
15. Haux J. *Med Hypotheses*, 1999; **53**:543-548.
16. Duke's Phytochemical and Ethnobotanical Databases. U.S. Department of Agriculture, Agricultural Research Service. 1992-1996.
17. Abubacker M N, Kamala Devi P. *European journal of pharmaceutical and medical research*, 2015; **2(5)**:1779-1787
18. Sarada K, Jothibai MR, Mohan VR. *International Journal of Chem Tech Research*, 2011; **3(3)**:1548-155.
19. Manju Madhavan. *International Journal of Pharmacognosy and Phytochemical Research*, 2015; **7(5)**:991-994.
20. Sivakumar SR. *International Journal of Advanced Research*, 2014; **2**:174-183.