ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

IDENTIFICATION OF BIOACTIVE COMPOUNDS EXTRACTED FROM BARKS OF *KANDELIA RHEEDIIA* AND EVALUATION OF *IN VITRO* ANTIDIABETIC POTENTIAL

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ABSTRACT

The present investigation was aimed to analyze the bioactive compounds of a therapeutically effective plant *Kandelia rheedii* bark and evaluation of antidiabetic potential by using *in vitro* assays. By soxhlet extraction, bioactive compounds were extracted followed by evaluation of physical characteristics, percentage extractive value, phytochemical screening and GC-MS analysis of methanolic extract of *Kandelia rheedii* and methanolic extract was evaluated for antidiabetic activity by employing standard *in vitro* techniques. The analysis provided seven different compounds like Phenol, 2, 4-bis (1,1-dimethyl ethyl)- (21.4%), Flavone (100%), 4H-1-benzopyran-4-one,7-hydroxy-2phenyl (25.2%), 4H-1 Benzo-pyram-4-one, 7-hydroxy-2-phenyl (23.8%), 5,8 Octadecadienoicacid, methyl ester-(58.5%), Coumarine, 3-(2,4-dinitrophenyl) (93.8%) and Elaidic acid, isopropyl ester (26.4%). *In vitro* assays confirm that methanolic extract of *Kandelia rheedii* exhibited antidiabetic activity. Phytochemical screening and GC/MS analysis can help to implement quantitative estimation with the help of markers and can be helpful for pharmaceutical applications. This study provides scientific evidence that *Kandelia rheedii* have antidiabetic efficacy.

Keywords: *Kandelia rheedii,* Rhizophoraceae, Phytochemical screening, GC/MS analysis, Mangrove plant, Antidiabetic, *In vitro* assays.

1. INTRODUCTION

Diabetes mellitus (DM) is set of metabolic disorder with a common feature of high blood glucose level often genetic in nature but can be developed due to life style and habits. Currently available anti-diabetic drugs possess severe side effects such as risk of hypoglycaemia. Due to the long history in folklore medicine, medicinal plants have not escaped the attention of today's pharmaceutical chemists. Medicinal plants are potential pharmacies grown in the wild and have been co-existed and co-evolved alongside human civilizations since the beginning of life on Earth. Since ancient times, human life has been revolving around plants as they were used for their curative nature to alleviate human pain and have been the focal point of many researchers since the dawn of medicine. The importance of traditional medicines has been well understood by the pharmaceutical industry since the discovery and successful development of aspirin from the symbolic Willow tree [1]. Mangrove is a shrub or small tree that grows in coastal brackish or saline waters in muddy or rocky soils. Mangroves are halophytes, being salt tolerant, they can quickly adapt themselves in harsh coastal conditions [2]. Currently, the word 'mangrove' encompasses 84 species from 24 genera and 16 families. However, only 70 species out of the 84 are classified as true mangroves while the rest as mangrove associates [3].

Kandelia rhedii, alternatively known as *K. candel*, is a mangrove plant found abundant in Indian subcontinent-especially in Bengal deltaic region. It falls into the family named Rhizophoraceae. It is a small evergreen tree and heights up to 6 m and much branched. Leaves of this plant are imparipinnate; leaflets range between 3 to 5 per leaf. Leaves are firm, 3.8-6.3 cm long, distant, alternate and suborbicular. The plant has small flowers-pale yellow in axillary panicles and shorter than the leaves. Pods are 3.8-10 cm long, lanceolate [4, 5]. *Kandelia rheedii* (locally known as Guria or Rasunia) is a well known herbal cure to tuberculosis. Several small molecules, for example Skyrin, Fusaric acid and Emodin, from the plants have been reported up till now. Skyrin, a fungal bisanthroquinone, exhibits functional glucagon antagonism by uncoupling the glucagon receptor from adenylate cyclase activation in rat liver membranes Fusaric acid is a picolinic acid

derivative. It is typically isolated from various Fusarium species, and has been proposed for a various therapeutic applications. Fusaric acid is an important antibacterial agent and can also be used to kill cancer cells. It thus can be used as a biocontrol agent. Emodin is a purgative resin, 6 - methyl - 1, 3, 8 - trihydroxyanthraquinone. Emodin is being studied as a potential agent that could reduce the impact of type2 diabetes [6].

The premier steps to utilize the biologically active compound from plant resources are extraction, pharmacological screening, isolation and characterization of bioactive compound, toxicological evaluation and clinical evaluation. Standardization refers to the body of information and control necessary to product material of reasonable consistency. This is achieved through minimizing the inherent variation of natural product composition through quality assurance practices applied to agricultural and manufacturing processes. For systematic approach the objective of this paper was to perform phytochemical studies addressing extracting and identifying bioactive compounds from barks of *Kandelia rheedii* for the standardization.

2. MATERIAL AND METHODS

2.1. Chemicals, reagents and plant materials

All the solvents used were of analytical reagent purity grade. The barks of *Kandelia Rheedii* W. & A. were collected in the month of March from the coastal region of Bhadrak situated near Cuttak (Odhisa). These were identified and authenticated by Dr. S. N. Dwivedi (HOD) and voucher specimens were deposited in the herbarium of the Department of Botany, Janata PG College, A.P.S. University, Rewa (M.P.). The barks were washed, shade dried, pulverized into moderately coarse powder and stored in airtight container for further use.

2.2. Extraction

The barks were washed, shade dried and pulverized into moderately coarse powder using hand grinder. Powdered barks were weighed and packed in soxhlet apparatus. The powdered bark was defatted with petroleum ether (40-60°C) for about 09 hrs and complete defatting was ensured by placing a drop from the thimble on a filter paper which did not exhibit any oily spot. The defatted material was removed from the soxhlet apparatus and air dried to remove last traces of petroleum ether. The defatted material was subjected to extraction by methanol as solvent. The extracts were collected in a tarred conical flask. The solvent was removed by distillation. Last traces of solvent were removed under vacuum. The extract obtained with each solvent was weighed to a constant weight and percentage w/w basis was calculated. The obtained crude extract was stored in dark glass bottles for further processing. Yield of the extract obtained was calculated by formula as mentioned below:

Extractive yield value = (Weight of concentrated extract/Weight of plant dried powder) × 100

2.3. Preliminary phytochemical investigations

Qualitative chemical tests of methanolic extracts were conducted to identify the various phytoconstituents [7,8].

2.4. GC/MS Analysis

The methanolic extract components were determined using a percent relative peak area. A temporal identification of the compounds was completed based on the comparison of their relative retention time and mass spectral data with those of the NIST, WILLY Library data of the GC/MS system [9]. The injector and MS transfer line temperature was adjusted at 280°C. The oven temperature was programmed initially at 40°C (for 3 minutes) to 280°C final at an increasing rate of 5°C/minutes (for 5 minutes).

2.5. Glucose uptake in yeast cells

Glucose uptake assay by using yeast cells was performed according to the method of Cirillo et al., [10]. The commercial baker's yeast in distilled water was subjected to repeated centrifugation (3,000×g, 5 min) until clear supernatant fluids were obtained and 10% (v/v) of the suspension was prepared in distilled water. Various concentrations of methanolic extract of *Kandelia Rheedii* (50 to 250µg/mL) were added to 1mL of glucose solution (5 mM) and incubated together for 10 min at 37°C. Reaction was started by adding 100 µL of yeast suspension followed by vortexing and further incubation at 37°C for 60 min. After 60 min, the tubes were centrifuged (2,500 × g, 5 min) and amount of glucose was estimated by using a spectrophotometer (UV 5100B) at 520 nm. Absorbance for the respective control was also recorded on the same wavelength. The percent increase in uptake was calculated by the formula: % increase in glucose uptake = {(Abs. of control - Abs. of sample)/ Abs. of control} × 100, where control is the solution having all reagents except the test sample. Metronidazole was used as standard drug.

2.6. Statistical Analysis

All the experiments were performed in triplicates (n=3) and the data are presented as the mean ± standard error. Differences between the means of the individual groups were analyzed using the analysis of variance procedure of SPSS software 20 Version (IBM). The significance of differences was defined at the $P < 0.05$ and $P < 0.01$ level.

3. RESULTS AND DISCUSSION

The outcomes of the physical characteristics and percentage extractive value of methanolic extract of *Kandelia rheedii* are tabulated in table 1. Percentage extractive value for methanolic extract was found to be 8.3%.

Phytochemical screening of the extracts showed the following results. Methanolic extract of *Kandelia rheedii* showed the presence of alkaloids, saponins, steroids, Phenolics, tannins, flavonoids, terpenes, glycosides, proteins and carbohydrates.

The structure of the compounds present in the metanolic bark extract was identified by GC-MS studies. The analysis provided seven different compounds like Phenol, 2, 4-bis (1,1-dimethyl ethyl)-(21.4%), Flavone (100%), 4H-1-benzopyran-4-one,7-hydroxy-2phenyl (25.2%), 4H-1 Benzopyram-4-one, 7-hydroxy-2-phenyl (23.8%), 5,8 Octadecadienoicacid, methyl ester-(58.5%), Coumarine, 3-(2,4-dinitrophenyl) (93.8%) and Elaidic acid, isopropyl ester (26.4%).

Mangrove plants are potential sources of biologically active chemicals that are discernible from their wide spread application in ethno pharmaceutical practices [11]. One of these attributes is the secondary metabolites produced by the mangroves which have been used traditionally by medicinal local practitioners due to their proved medicinal values [12]. Phytochemical analysis is a good technique to check the genetic variability present in plant species. Further GC/MS analysis helped to distinguish the phytoconstituents and to implement quantitative estimation with the help of markers and it helpful for pharmaceutical applications.

In yeast cells model, the methanolic extract of *Kandelia rheedii* at different concentrations (50µg-250µg) were subjected to *in vitro* glucose uptake assay using yeast as model. The percentage of glucose uptake in yeast cells by the extract was compared with Metronidazole standard drug. In glucose uptake assay *Kandelia rheedii* extract and standard showed dose dependant manner of activity *i.e.* as the concentration of sample increased even the percentage of inhibition also increases.

Table 1: Physical characteristics and percentage extractive value of methanolic extract of *Kandelia rheedii*

Extract	Colour	Odour	% Extractive value
Methanolic	Dark brown	Characteristic	8.3%

Table 2: Preliminary phytochemical screening of methanolic extract of *Kandelia Rheedii*

S. No	Phytoconstituents	Methanolic extract
1	Alkaloids	++
2	Saponins	+
3	Steroids	+
4	Phenolic compounds	+
5	Tannins	+++
6	Flavonoids	+
7	Terpenoids	++
8	Glycosides	+
9	Protein & Amino acids	+
11	Carbohydrate	+

+++ Highly present, ++ Moderately present, + Faintly present

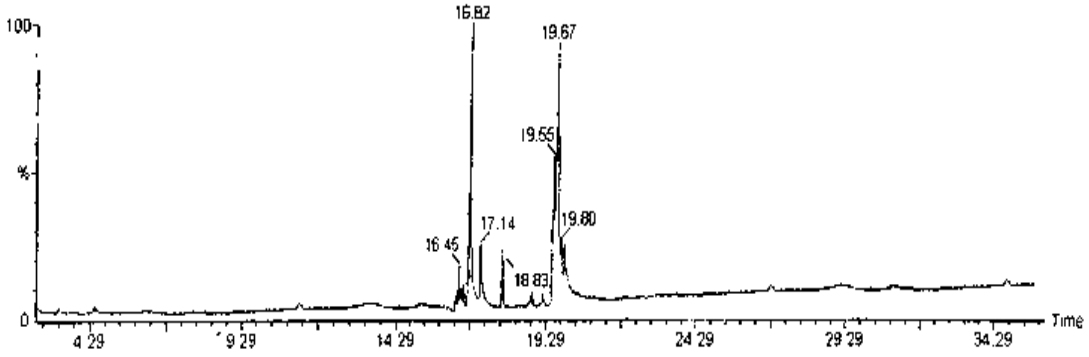

Fig. 1: GC-MS analysis of *Kandelia rheedii* bark extract

Table 3: GC-MS analysis of *Kandelia rheedii* bark extract

Peak	RT	Name of the compound	Molecular formula	Molecular weight	Peak area (%)
1	16.45	Phenol,2,4-bis(1,1-dimethyl ethyl)	$C_{14}H_{22}O$	209.332	21.4%
2	16.82	Flavone	$C_{15}H_{10}O_2$	222.243	100%
3	17.14	4H-1-Benzopyran-4-one,7-hydroxy-2-phenyl	$C_{15}H_{10}O_3$	238.24	25.2%
4	18.83	4H-1-Benzopyran-4-one, 3-hydroxy-7-methoxy-2-phenyl	$C_{16}H_{12}O_5$	284.267	23.8%
5	19.55	5,8-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294.479	58.5%
6	19.67	Coumarine, 3-(2,4-dinitrophenyl)	$C_{15}H_7N_3O_8$	357.231	93.8%
7	19.80	Elaidic acid, isopropyl ester	$C_{21}H_{40}O_2$	324.549	26.4%

Table 4: Percentage of glucose uptake in yeast cells treated with *Kandelia rheedii* extract

Samples	Concentration(µg/ml)	Inhibition (%)	IC50(µg/ml)
Standard	50µg	51.47±1.00*	48.56 µg
	100 µg	58.20±1.05*	
	150 µg	62.48±0.50*	
	200 µg	65.70±0.28*	
	250 µg	69.74±0.25*	
Methanol extract	50µg	47.21±1.00*	102.20 µg
	100 µg	48.92±0.62*	
	150 µg	55.54±0.47*	
	200 µg	58.31±1.14*	
	250 µg	61.36±0.73*	

*Results are expressed as Mean±SE (n=3); * significant at the P < 0.01.*

The mechanism of glucose transport across the yeast cell membrane has been receiving attention from *in vitro* screening method for hypoglycaemic effect of various compound/medicinal plants [13]. The amount of glucose remaining in the medium after a specific time serves as an indicator of glucose uptake by yeast cells [14]. The methanolic extract of *Kandelia rheedii* promoted the uptake of glucose across the plasma membrane of yeast cells. The glucose uptake at an initial concentration of methanolic extract of *Kandelia rheedii* was comparable to that of known drug metronidazole. However, the effect of metronidazole on glucose uptake by the yeast cells at 250 µg/ml glucose concentration was a bit higher as compared to that of methanolic extract. The results indicate that methanol extract of *Kandelia rheedii* showed appreciable antidiabetic activity in performed *in-vitro* assays as compared to the standard. On the other hand, an inverse relationship to the molar concentration of glucose was observed, when glucose uptake by yeast cells was compared among 50 to 250 µg/mL for the same amount of methanolic extract. From the results it is concluded that the lower the concentration of glucose in the solution, the higher the uptake by yeast cells. Hence, glucose transport occurs only if the intracellular glucose is effectively reduced (utilized). The present data, suggest that the methanolic extract is capable of enhancing glucose uptake effectively, which in turn suggest that it is capable of enhancing effective utilization at 250µg/mL concentration, thereby controlling blood glucose level as also suggested by other reports. It therefore, justifies the claim of its use as an antidiabetic drug in traditional medicine comparatively, in the present study; the increased ability of the sample to absorb glucose may also be attributed to the dietary fiber (insoluble and soluble fibers) present in the methanolic extract.

4. CONCLUSION

In recent years, screening of mangrove plants for a variety of biological activities is gaining importance. Mangroves are biochemically unique, producing a wide array of novel natural products. The findings indicate that *Kandelia rhedii* are prolific producers of bioactive metabolites and have antidiabetic efficacy. They have the potential of producing compounds which have anticancer, antiviral, antibacterial, antifungal activities and some of them exhibit strong potential to be developed as a new drug. Efforts are needed to take these compounds forward for drug development.

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