Biosciences, Biotechnology Research Asia Vol. **6**(1), 293-296 (2009)

Isolation and characterization of aromatic amide from methanolic extrat of root of *Carissa carandas* Linn.

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(Received: November 26, 2008; Accepted: December 30, 2008)

ABSTRACT

Carissa carandas Linn (Family: Apocynaceae) commonly known as Karaunda, is a large evergreen shrub with a short stem, glabrous shrub found almost through out India. All the parts of the *Carissa carandas linn* were reputed in indigenous medicine for various kinds of diseases and disorders. The objective of the work is to study the chemical constituents of benzene extract of root of *Carissa carandas* Linn. The fresh root of the plant is used internally as anthelmentics, antipyretic, stomachic and antiscorbutic. Phytochemical investigation conducted on root revealed the presence of carbohydrates, alkaloids, flavanoids, saponins, steroids and fatty acids. The aromatic amide was detected in benzene extract and methanol extract of the root. The extracts were carried out by soxhlet extractor and the yield of benzene extract was 2.5%. The TLC study of methanolic extract showed the presence of 4 spots. The three spots were identified as aromatic amides. The present study deals with isolation characterization of aromatic amide (M_3) isolated from methanolic extract of root of *Carissa carandas linn.* The four compounds were separated by column chromatography using benzene: ethyl acetate: chloroform (4:2:1). The TLC study of separated compounds with Rf value 0.85 (M_1), 0.73 (M_2), 0.68 (M_3) and 0.65 (M_4). From UV, IR, ^{1}HNMR and LC-Mass spectral analysis of the compound M_3 .It could be characterized as aromatic amide.

Key words: *Carissa carandas*, methanolic extract, aromatic amide, TLC, column chromatography, Rf value, IR,^{1}HNMR and LC-Mass.

INTRODUCTION

Carissa carandas Linn (Family: Apocynaceae) commonly known as Karaunda, is a large evergreen shrub with a short stem, glabrous shrub found almost through out India[1].

C.carandas Linn is a large evergreen shrub with short stem and strong thorns in pairs, bark light grey,scaly;leaves simple,opposite,elliptic or obovate,shortly mucronate, glabrous shining and coriaceous; flowers white, in pubescent terminal corymbose cymes; fruit ellipsoid or globose berry, purplish black when ripe enclosing two or more seeds[2]. The root has reputation of bring better stomachic. Used in the konkan, pounded with horse urine, lime juice and camphor as remedy for itching.[3-5] the presence of cardio tonic activity of water soluble fraction has been attributed to the presence of glycosides of odoroside H. Presence of alkaloids is also reported in root and stem bark.[4]

MATERIAL AND METHODS

The fresh root samples of *carissa carandas linn* was collected, dried and cut into pieces, crushed into powder then passed through sieve no 40 to obtain uniform particles and extracted with the solvents of increasing polarity6, Petroleum ether, benzene, chloroform, ethyl acetate and methanol for 48 hrs in fifty batches of 100gms for each batch by successive solvent extraction method. The extracts were collected to dryness in a rotary evaporator under reduced pressure and controlled temperature (50-60°). After drying the extracts were weighed.

Thin Layer Chromatographic (TLC) of Methanolic Extract

The crude Methanolic extract was subjected to TLC studies using precoated silica gel GF^{254} Plates of 0.2 thickness[7]. A suitable mobile phase was developed consisting of benzene: ethyl (0.2g viewed under UV light and by spraying with dilute sulphuric acid. TLC study of methanolic extract showed the presence of 4 spots.

Column chromatography of methanolic extract

The methanolic extract was chromatographed in a column with a aluminium oxide neutral built in petroleum ether and eluted with benzene: ethyl acetate: chloroform (4:2:1), four fractions were collected and concentrated.Fraction-1 upon concentration yielded reddish semisolid mass (0.2g) and named them as M_1.Similarly fractions 2, 3, and 4 are concentrated and yielded, 0.25 g, 0.3 g and 0.35 g respectively).They were labeled as M_2, M_3,and M_4 respectively . The collected fractions are subjected to thin layer chromatography using the solvent system benzene: ethyl acetate: chloroform (3:2:1), spraying the developed plates with dilute sulphuric acid yellow color spots were observed. The Rf value of four compounds were calculated as M_1 R_f value =0.85, M_2 R_f value = 0.73, M_3 R_f value= 0.68, and M_4 R_f value = 0.65 All the four compounds were subjected for anti fungal activity the compound M_3 was showed the moderate anti fungal activity hence the compound M_3 was subjected for spectral analysis for further studies to know the chemical constituent responsible for the activity.

Chemical and spectroscopic analysis of Compound M_3

The compound M_3 was examined for there colour, odour, nature, melting point and solubility. The melting point was determined by open capillaries of glass and they are uncorrected. The UV spectrum of the compound was recorded by using UV spectrophotometer. The IR spectrum was recorded in KBr pellet using FTIR spectrophotometer. The ^{1}HNMR of the compound was recorded in $CDCl_3$ using Bruker NMR spectrophotometer. The data obtained are given in Table 1.

Antifungal activity of aromatic amide of root of Carissa carandas

The crude aromatic amide isolated from benzene extract were tested for antifungal activity by cup plate method[8] at 1% and 2% concentration in dimethyl sulphoxide and fluconazole was used as standard drug. The photo agar media was used for this work[8]. The test organisms used were (*A. Niger* and*, A Fumigatus*).The zone of inhibition was measured in mm Table 2.

RESULTS AND DISCUSSION

From benzene extract of root of *carissa carandas* linn aromatic amide was separated by column choromatography.The table I and II shows the results of spectral analysis and anti-fungal activity of M_3 compound.

TLC studies of methanolic extract showed four spots and developed yellow colour with dilute sulphuric acid. The aromatic amide with R_f value of M_3 (0.68) was isolated by preparative TLC.

In the spectroscopic analysis of M_3 compound showed an absorption bands. In IR spectrum KBr) cm^{-1} .Table I suggested presence of 3460 cm^{-1} (hydrogen bonded NH2 group), the weak band around 3000-3100 cm^{-1} (aromatic stretching), 2300-2919 cm^{-1} (C-H stretching of the CH_2 and CH_3 groups), 1706 cm^{-1} (C=O group),1652 cm^{-1} (N-H bending and C=N) and 1608 cm^{-1} (due to C= C) .

The ^{1}HNMR of the compound M_3 showed the characteristics signals as below The major peaks

Table 1: Spectroscopic analysis of compound M_3

Compound	Spectrum	Characteristics Peaks
M_3	UV Spectrum	λmax=310nm
	IR Spectrum (KBr) Cm^{-1}	3460(NH2 group), 3000-3100 (aromatic stretching), 2300-2919 (C-H stretching of the CH_2 and CH_3 groups) 1706C=O group), 1652 (N-H bending and C=N), 1608 (C=C).
	^{1}HNMR($CDcl_3$)	0.6– 4.3 δppm(presence of CH_2 and CH_3groups) 5.5 δppm (NH_2 group) 7.2–7.7 δppm(presence of aromatic ring)
	LC-Mass	m/z 413.5 (the basic peak)

Table 2: Antifungal activity of compound M_3

Microorganism	Zone of inhibition in mm			
	B_5(compound)		(Fluconazole)	
	1%W/W	2%W/W	1%W/W	2%W/W
A. Niger	16	17	16	18
A Fumigatus s	17	18	18	19

observed in the ^{1}HNMR spectrum at δ0.6-4.3 indicates the presence of CH_2 and CH_3 groups. The peak at 5.5 may be due to NH_2 group and the peak at δ 7.2–7.7 may be due to aromatic proton indicating the presence of aromatic ring. The IR and ^{1}HNMR spectral studies of component M_3 suggests the presence of aromatic ring, amino group, carbonyl group, and methylene and methyl group. Hence, the compound M_3 may contain an aromatic with aside chain having CH_2 and CH_3 groups and containing amide group. Hence the compound M_3 is an aromatic amide. In contrary, the mechanism of amides will not depend on chain length and number of double or triple bonds. It has been hypothesized to produce its effects through conjugation with peptides and proteins of surface membranes thereby increase the permeability of the membrane and effective against all types of microbes. This could be the reason behind the antifungal activity of M_3 compound. The phytochemical investigation and spectral analysis of compound M_3 has shown the presence of aromatic amide in Methanolic extract. The aromatic amide could be the reason for its effectiveness as antifungal activity.

The LC-Mass spectrum of compound B_5 showed the base peak at m/z 803.6.The molecular peak not recorded. The antifungal activity of the compound B_5 because of aromatic amide.

ACKNOWLEDGEMENTS

Authors are greatly thankful to Dr.R.H.Udapi Professor in department of chemistry N.E.T.College of Pharmacy Raichur for his help in interpretation spectra.

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