Asian Journal of Chemistry Vol. 22, No. 2 (2010), 1005-1008

Studies on Aromatic Amide of Root of *Carissa caranadas* Linn

MALLIKARJUN MALIPATIL*, RAMESH CHANDRA† and H. RAMESH‡
Department of Pharmacognosy, Karnataka College of Pharmacy
Manhalli Road, Bidar-585 403, India
E-mail: mhp1232007@rediffmail.com

The objective of the work is to study the chemical constituents of benzene extract of root of *Carissa carandas* Linn. 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 %. TLC study of benzene extract showed the presence of 5 spots, out of which one spot was identified as aromatic amide. The present study deals with chemical investigation of benzene extract of root of *Carissa carandas* Linn. The five compounds were separated by column chromatography using benzene:ethyl acetate:chloroform (4:2:1). TLC study of separated compounds with R_f value 0.45 (B_1), 0.58 (B_2), 0.93 (B_3), 0.62 (B_4) and 0.59 (B_5). From UV, IR, ^{1}H NMR and LC-MS analysis of the compound B_5, it could be characterized as aromatic amide.

Key Words: *Carissa carandas* Linn., Benzene extract, Aromatic amide.

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].

†Dr. B.R. Ambedkar Center for Biomedical Research Delhi University, Delhi-110 006, India.
‡R.R.K College of Pharmacy, Naubad, Bidar-585 402, India.

EXPERIMENTAL

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 polarity[6], petroleum ether, benzene, chloroform, ethyl acetate and methanol for 48 h in 50 batches of 100 g for each batch by successive solvent extraction method. The extracts were collected to dryness in a rotary evaporator under reduced pressure and controlled temperature (50-60º). After drying the extracts were weighed.

Thin layer chromatographic (TLC) studies of the fatty acids of benzene extract: The crude benzene 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 acetate:chloroform (4:2:1). Which gave maximum resolution of the spots. The spots were viewed under UV light and by spraying with dilute sulphuric acid. TLC study of benzene extract showed the presence of 5 spots.

Column chromatography of benzene extract: The benzene 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) five fractions were collected and concentrated. Fraction-1 upon concentration yielded reddish semisolid mass (0.3 g) and named them as B_1. Similarly fractions 2, 3, 4 and 5 are concentrated and yielded (0.2, 0.25, 0.3 and 0, 2 g, respectively). They were labeled as B_2, B_3, B_4 and B_5, 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 colour spots were observed. The R_f value of five compounds were calculated as B_1 R_f value = 0.45, B_2 R_f value = 0.58, B_3 R_f value = 0.93, B_4 R_f value = 0.62 and B_5 R_f value = 0.55. All the five compounds were subjected for antifungal activity the compound B_5 was showed the moderate anti fungal activity hence the compound B_5 was subjected for spectral analysis for further studies to know the chemical constituent responsible for the activity.

Chemical and spectroscopic analysis of compound B_5: The compound B_5 was examined for its 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. ^{1}H NMR of the compound was recorded in $CDCl_3$ using Bruker NMR spectrophotometer.

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 potato agar media was used for this work[8]. The test organisms used were (*A. niger* and *A. fumigatus*).

RESULTS AND DISCUSSION

From benzene extract of root of *Carissa carandas* Linn aromatic amide was separated by column choromatography. Tables 1 and 2 shows the results of spectral analysis and antifungal activity of B_5 compound.

TABLE-I
SPECTROSCOPIC ANALYSIS OF COMPOUND B_5

Compound	Spectrum	Characteristics peaks
B_5	UV spectrum	λ_{max} = 320 nm
	IR Spectrum (KBr) cm^{-1}	3442 (NH_2 group), 3000-3100 (aromatic stretching), 2400-2923 (C–H stretching of the CH_2 and CH_3 groups), 1732 (C=O group), 1652 (N–H bending and C=N), 1600 (C=C).
	^{1}HNMR ($CDCl_3$)	0.6-3.0 δppm (presence of CH_2 group), 3.5-4.5 δppm (presence of CH_3 group), 5.1 C δppm (NH_2 group), 6.7-7.7 δppm (presence of aromatic ring)
	LC-mass	m/z 803.6 (the basic peak)

TABLE-2
ANTIFUNGAL ACTIVITY OF COMPOUND B_5

Microorganism	Zone of inhibition in mm.			
	B_5 compound		Fluconazole	
	1 % w/w	2 % w/w	1 % w/w	2 % w/w
A. niger	14	16	16	18
A. fumigatus	15	17	17	18

TLC studies of benzene extract showed five spots and developed yellow colour with dilute sulphuric acid. The aromatic amide with R_f value of B_5 (0.59) was isolated by preparative TLC.

In the spectroscopic analysis of B_5 compound[9] showed an absorption bands. In IR spectrum KBr, cm^{-1}. Table-1 suggested presence of 3442 cm^{-1} (hydrogen bonded NH_2 group), the weak band around 3100-3000 cm^{-1} (aromatic stretching), 2923-2400 cm^{-1} (C–H stretching of the CH_2 and CH_3 groups), 1732 cm^{-1} (C=O group), 1652 cm^{-1} (N–H bending and C=N) and 1600 cm^{-1} (due to C= C).

The major peaks observed in the ^{1}H NMR spectrum at δ 0.6-3.0 and at δ 3.5-4.5 indicates the presence of CH_2 and CH_3 groups. The peak at δ 5.1 may be due to NH_2 group and the peak at δ 6.7-7.7 may be due to aromatic proton indicating the presence of aromatic ring. The IR and ^{1}H NMR spectral studies of component B_5 suggests the presence of aromatic ring, amino group, carbonyl group and methylene and methyl group. Hence, the compound B_5 may contain an aromatic with aside chain having CH_2 and CH_3 groups and containing amide group. Hence, the compound B_5 is an aromatic amide[10]. 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 B_5 compound. The phytochemical investigation and spectral analysis of compound B_5 has shown the presence of aromatic amide in benzene 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

The authors are greatly thankful to Prof. R.H. Udapi, Department of Chemistry, N.E.T. College of Pharmacy, Raichur for his help in spectral interpretation.

REFERENCES

1. K.R. Kirtikar, L.M. Basu and M.D. Basu, Indian Medicinal Plants, Allahabad, India pp. 1546-1548 (1984).
2. P.S. Vaidyratnam, Variers Arya Vaidya Sala Kohakal, Indian Medicinal Plants Orient Longam Limited, pp. 386-389 (1984).
3. A. Rajashekharan, A. Jeyasudha and V. Kalpana, *Indian J. Nat. Prod.*, **15**, 27 (1999).
4. The Wealth of India, Vol 3, Revised Edition, Publication and Information Directorate, New Delhi: CSIR; (1992).
5. A.K. Nadkarni and K.M. Nadkarni, Indian Materia Medica, Bombay, Popular Book Depot, p. 266 (1954).
6 C.K. Kokate, Practical Pharmacognosy, Vallabh Prakashan, New Delhi, edn. 4, p.107 (1994).
7. E.J. Sathl, Thin Layer Chromatography, Laboratory Handbook, Sringer Verlag, Berlin, edn. 2, p. 421 (1962).
8. W.B. Hugo and A.D. Russel, Pharmaceutical Microbiology, Blackwell Scientific Publications, London, edn. 5, p. 273 (1992).
9. M. Silverstein, C. Bassler and T.C. Morril, Spectrophtometric Identification on Organic Compounds, John Wiley and Sons, New York, edn. 4, p. 104 (1981).
10. G.W. Gokel and Dean, Handbook of Organic Chemistry, McGraw Hill Publications, edn. 2, p. 6.21 (2000).

(*Received*: 26 November 2008; *Accepted*: 10 October 2009) AJC-7938