ScienSage

Journal of Advanced Scientific Research

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

ISSN
0976-9595
Research Article

SPECTROGRAPHIC ANALYSIS AND *IN VITRO* STUDY OF ANTIBACTERIAL, ANTICANCER ACTIVITY OF AQUEOUS ETHANOLIC FRUIT EXTRACT OF *CARISSA CARANDAS* L.

Muniswamy David* and Gururaja Karekalammanavar

Laboratory of environmental toxicology and molecular biology, Department of PG Studies in Zoology,Karnatak University, Dharwad, Karnataka,
**Corresponding author: mdavid.kud@gmail.com*

ABSTRACT

Fruits are the richest source of nutrients and minerals. *Carissa carandas* belongs to the Apocynaceae family and widely spread throughout India. Spectrographic methods are most reliable, for the analysis of phytochemicals. *Carissa carandas* fruits are plentiful sources of phytochemicals and possessing beneficial biological activities. The present work was first to focus on analysing of such phytochemicals in aqueous ethanolic fruit extract (AEE) using UV-Visible and Fourier transform infrared spectroscopy (FTIR). The fingerprint of the UV-spectra showed maximum absorption peak at 270 nm and FTIR helps in the recognition of functional groups and assignments. Furthermore, *in-vitro* studies showed that AEE possesses lowest MIC value 0.3125 mg/ml and induces cytotoxicity at 800µg/mL on HeLa cancer cells. The study concludes that, regular intake of fruits in daily diet suggested with reduced risks of infectious diseases and cancer.

Keywords: *Carissa carandas*, UV-VIS, FTIR, MIC, anticancer

1. INTRODUCTION

For decades, the application of natural herbal products has drawn much interest as they could hold the therapeutic response and are promising prospect to be developed as pharmaceutical products [1]. The plant *Carissa carandas* belongs to the Apocynaceae family and widely spread throughout India. The plant has been utilized as a traditional medicinal plant over thousands of years in the Ayurvedic, Unani, and homeopathic system of medication. The plant possesses various phytochemicals which have been used for enthnomedicine in the treatment of diarrhea, stomachic, anorexia, mouth ulcer, scabies and epilepsy [2-4]. Phytoconstituents like triterpenoids, carissol, uroslic acid, lupeol, and β-sitosterol has been accounted to be present in the fruit of this plant [5-6]. Previous studies were reported that, these constituents as an anti-inflammatory, anti-helminths and nephrotoxicity [5, 7-8]. Sulaiman *et al.,* [9] found that, the fruit extract of different solvent system exhibited good anticancerous activity on human ovarian carcinoma and lung cancer cells.

Spectrographic methods are most reliable, for the analysis of phytochemicals. Ultraviolet-visible spectroscopy is routinely used to detect the characteristic wavelength of the extract. Fourier transform infrared spectrometry (FTIR) has been suggested as an alternative technique for taking a fingerprint of functional groups in fruit extract and also assists in structural interpretation of the compounds [10-11] and thus affording the new panoramas for the investigation of phytochemicals. To the best of our knowledge, the present work was first to report on the spectrographic analysis and their effectiveness as antibacterial and anticancer agent. Hence, the present investigation was aimed for spectrographic analysis and *in-vitro* study of antibacterial and anticancer activity of the aqueous ethanolic fruit extract of *Carissa carandas*.

2. MATERIALS AND METHODS

2.1. Collection of plant material

Healthy, fruits (dark purple color) were collected from the Karnatak University campus, Dharwad, Karnataka, India, during May 2015. World Geodetic system (WGS84) of Karnatak University, Dharwad is 15° 26′ 28.5″ N, 74° 59′ 2.1″ E 15.44125, 74.983917 with annual average rainfall 838mm, average temperature ranging from 19.4°C to 30°C.

2.2. Washing method

The bacterial and fungal spores on the fruits accelerated the pace of deterioration; hence washing the fruits is a crucial measure in keeping them clean. The fruits were washed in a diluted vinegar bath (1:3) and twirl them dry in a paper lined salad spinner. The fruits were stored in a container lined with paper towels, keeping the lid open a little to permit moisture to escape [12].

2.3. Reagents

Vinegar was purchased from local market Dharwad, Petroleum ether and Ethanol was purchased from Sisco

Research Laboratory (SRL), Potassium bromide (FT–IR grade), LB-broth media, Dulbecco's Modified Eagles Medium, Fetal Bovine Serum, Penicillin and Streptomycin, Dimethyl sulfoxide and the MTT assay kit was purchased from Himedia. The HeLa cancer cells were procured from NCCS, Pune and were maintained at BSRC, Belgaum.

2.4. Preparation of Extract

The washed fruits (50g) were separated from seeds and defatted with petroleum ether (500mL), filtered through two folded muslin cloth and the extracted residue was subjected to (50%) aqueous ethanolic (500mL) solution, raising up in shaking incubator (Remi CIS-24 Plus) for 48h. Filtered and concentrated in a laboratory hot air oven (40°C) to give a sticky reddish brown liquid (5mL) called aqueous ethanolic extract (AEE). The AEE was stored in a screw cap bottle at 4°C for further experiments.

2.5. UV-Visible spectroscopic analysis (UV-VIS)

An aliquot of the extract was diluted with distilled water and scanned using UV- Visible Spectrophotometer (Hitachi, U-3310) at a range of 200-800 nm, to detect the characteristic wavelength of the AEE.

2.6. Fourier Transform Infra Red Spectroscopic analysis (FTIR)

FTIR spectroscopy (Thermo scientific Nicolet 6700) was used that was equipped with a deuterated triglycine sulfate (DTGS) as a detector. IR spectra were recorded in the 500-4000 cm^{-1} range with a resolution of 4 cm^{-1}. The method for sample preparation was followed by Yan *et al*., [13]. Briefly, dried KBrapowder (FT-IR grade) was weighed (200mg) and formed in two pieces of slender, transparent blank KBr pellets (5mm in diameter and ~1mm thickness). The AEE (2µL) was coated on the KBr pellets to form thin liquid film for IR analysis. The sample measurements were replicated 3 times with 2 scans each for aggregate of 6 spectra, and then the average chart was submitted as a last sample spectrum. The spectra interpretation was produced in accordance with Stuart, 2004 [14].

2.7. Minimum Inhibitory Concentration (MIC)

The MIC of AEE was tested against four strains of bacteria: two Gram positive species (*Streptococcus aureus* and *Enterococcus faecalis*) and two Gram negative species (*Escherichia coli* and *Klebsiella pneumonia*). "Broth micro dilution" method was employed for determination of MIC values [15]. AEE was dissolved in sterile distilled water (no activity against a test organism) to make 10mg/mL final concentration. Two fold serially diluted extract was added to broth media and bacterial culture of 100µl was added to each micro centrifuge tube (Tarsons) following negative (only bacterial suspension) and positive control (bacterial suspension with drugs). Single set of tubes was incubated at 37°C for 24h and another was kept at 4°C for comparing the turbidity. The lowest concentration of the extract in the tube that showed no turbidity after incubation was considered as MIC values. The tube, which showed the turbidity interpreted as visible growth of bacteria.

2.8. Anticancer activity

The HeLa cancer cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), Penicillin (100 U/ml), and Streptomycin (100 mg/cc) in a humidified atmosphere of 5% CO_2 incubator at 37°C. Cells (1 X 10^5/well) were seeded in 100 ml of DMEM medium/well in 96-well plates and incubated in a CO_2 incubator at 37°C for 48h. Confirming the confluence (~80%), the cells were treated with various concentrations of AEE (100-1000µg/mL) and then final volume was made to 200µl with 0.1% Dimethyl sulfoxide (DMSO) and incubated in a CO_2 incubator at 37°C for 48h. After incubation, media containing extract was taken out and rinsed with phosphate buffered saline (pH 7.4), then 20 µl/ well (5 mg/ml) of 0.5% 3-(4, 5-dimethylthiazol-2-yl) -2, 5-diphenyl–tetrazolium bromide (MTT) solution was added and the plates were filled with aluminium foil and continued in a CO_2 incubator for 4h at 37°C until a purple precipitate was visible. Then, the media were removed carefully without disturbing cells and do not rinse with PBS. 150µl DMSO (MTT solvent) was added to dissolve the purple precipitate. The Absorbance of the purple color was read at 570 nm with a microplate reader (Bio- Rad, Richmond, CA) using wells containing cells without sample as negative control and with samples as positive control (5-Florouracil). Measurements were performed in triplicates, and were determined graphically. The effect of the samples was expressed as the % cell viability, using the following formula:

% Cell viability = (absorbance of treated cells) /(absorbance of non treated cells) ×100

3. RESULTS AND DISCUSSION

3.1. UV-visible spectroscopic analysis

A UV-VIS spectrum of AEE (1:100 dilution) was taken at the 200-800 nm wavelength (fig. 1).

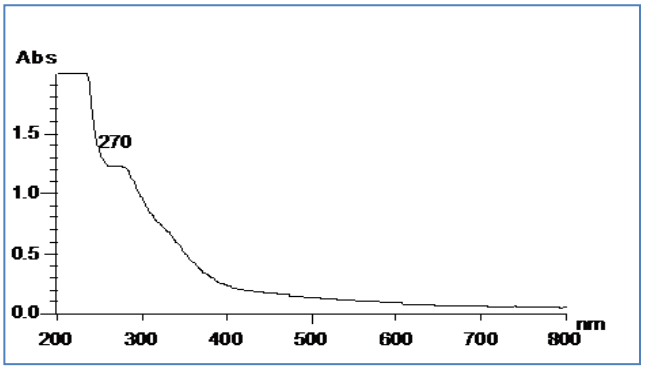


Fig.1: UV-Visible Spectra of AEE

The spectra showed the peak at 270 nm with 1.231 AU. The characteristic wavelength for phenolic compounds may lie

in the range 260-280 nm and the obtained peak may indicate the presence of phenolic compounds. Previous studies found the presence of phenolic compounds in the fruit [5-6]. Established on the analysis of UV-VIS spectra, one can say that the AEE contains phenolic compounds. This spectrum was evident in identifying the specific bioactive classes of molecule found in AEE.

3.2. Fourier Transform Infra Red Spectroscopic analysis

The fruit has been credited for various phytoconstituents. To analyze the phytochemicals found in AEE, we used complementary measurement by FTIR spectroscopy (fingerprints of functional groups).The IR spectra (fig.2) lies in the range of 4000-500 cm^{-1} and can be approximately divided into four regions and the nature of the group frequency may generally be determined by the region in which it is located [16].

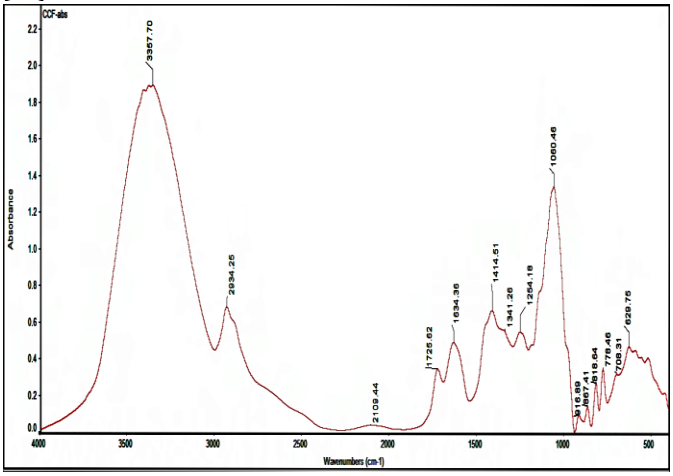


Fig.2: FT-IR spectra of AEE

The regions are generalised as X-H stretching (4000-2500 cm^{-1}), triple bond stretching (2500-2000 cm^{-1}), double bond stretching (2000-1500 cm^{-1}) and fingerprint region (1500-600 cm^{-1}). The broad peak at 3357.70 cm^{-1} corresponding to O-H stretching, that may due to the presence of phenols and alcohols. The band at 2934.25 cm^{-1} attributed as an alkanes that may bearing methylene asymmetric C-H stretching. The double bond region has a short band at 2109.44 cm^{-1} assigned an alkynes that may due to C=C stretching. The bands at 1725.62 cm^{-1} and 1634.36 cm^{-1}, corresponds the stretching vibration of (C=O) aldehydes and ketones. The movement of a group of atoms, or the bending or stretching bands of a particular bond shows many bands in the fingerprint region. The band at 1414.51 cm^{-1} attributed to =C–H in-plane bending may indicate the existence of alkenes. The two small bands at 1341.26 cm^{-1} and 1254.18 cm^{-1} represented as aromatic C-N stretching and may attribute as amines. The peak at 1060.46 cm^{-1} stands for carboxylic acids within the bending vibration of C-O-H. The bands between 916.89 cm^{-1} and 629.75 cm^{-1} assigned as out-of-plane C-H bending and may correspond to aromatic and halogen compounds. The spectra clearly evident for the presence of alcohols, phenols, aldehydes, ketones, carboxylic acids and halogen compounds. The chemical constituents were in compliance with other published reports [6, 15]. The spectrographic study may facilitate in analysing and classification of chemical constituents with their functional groups.

3.3. Minimum Inhibitory Concentration

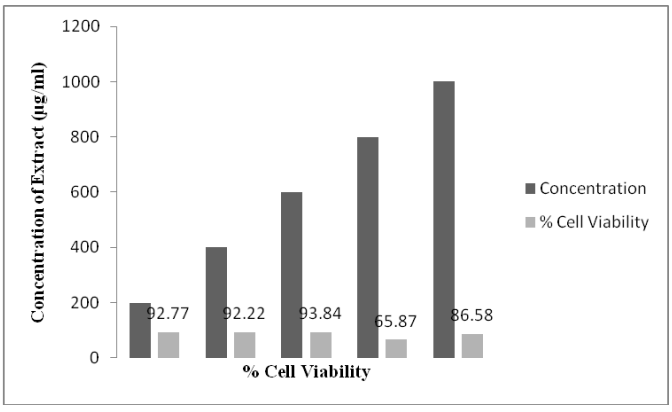
The lowest MIC value 0.3125 mg/ml was recorded against *S. aureus* and *K. Pneumonia* implies significant antibacterial activity of AEE. The extract has inhibited the growth of bacterial strains at low concentration and suggests the rich ability of the antibacterial activity of the extract (table 1). Mishra *et al.,* [17] also reported that, the fruit exhibited significant antibacterial activity. The results mean that the AEE is used as a better antibacterial agent.

Table 1: MIC recorded for AEE

Bacteria	*S.aureus*	*E. faecalis*	*E.coli*	*K. pnumoniae*
AEE (mg/mL)	0.3125	2.5	5	0.3125

3.4. Anticancer activity

Ethno-medically, *C. carandas* fruits are used as a remedy for curing various diseases as they are an excellent source of nutritional and minerals. The present study showed anticancer activity against the HeLa cancer cells (fig.3). The results deduce that, 65.87% of cancer cells were viable at concentration 800µg/ml. Previous studies were found that, the fruit has anticancer activity on breast cancer, human ovarian carcinoma and lung cancer cells [18]. The study clearly suggests that, the fruit can be applied as an anticancer agent in diagnosing the disease.



CCF: Carissa carandas fruit extract

Fig.3: Cytotoxicity effect of AEE on HeLa cancer cells

4. CONCLUSION

The study may demonstrate the complementary analysis of phytochemicals using UV-Vis and FTIR spectroscopy. FTIR spectra facilitate in analysis and also help in classification of phytochemicals with their functional groups. The aqueous ethanolic extract showed good antibacterial and anticancer activity. Therefore regular consumption of fruit is associated with reduced risks of infectious diseases and cancer. The future research can be carried out by isolating the specific bioactive compound with different solvents and there further characterization was needed.

5. ACKNOWLEDGEMENT

The authors were thankful to BSRC, Belgaum, for providing the laboratory facility to carry out the MIC and anti-cancer studies. Authors, greatly acknowledge the UPE-FAR-1 Program for financial assistance.

6. REFERENCES

1. Yakut Bint-e-Sadek1, Naiyyum Choudhury and Mohammad Shahriar. *International Journal of Pharmaceutical Research and Technology*, 2013; **5**; 97-105.
2. Begum S, Syed SA, Siddiqui BS, Sattar SA, Choudhary MI. *Phytochem Lett* ,2013; **6**:91-5.
3. Ganapaty S, Bharath CH, Laatsch H. *Int J Green Pharm*, 2010; **4**:186.
4. Hegde K, Joshi AB. *Indian J Exp Biol*, 2009; **47**:660-7.
5. Jayesh Dhodi B, Deepavali Thanekar R, Snehal Mestry N, Archana Juvekar R. *Journal of Acute Disease* ,2015:35-140.
6. Devmurari V, Shivanand P, Goyani MB, Vaghani S, Jivani NP. *Pharmacogn Rev,* 2009; **3**:375.
7. Anupama N, Madhumitha G, Rajesh KS. *Biomed Res Int,* 2014.
8. Mishra CK, Sasmal D, Shrivastava B. *Int J Drug Dev Res* , 2012;**4**:393-7.
9. Sulaiman SF, Wong ST, Ooi KL, Yusof SR, Muhammad T, Sifzizul T. Anticancer study of *Carissa carandas* extracts. Malaysia, 2008.
10. Socaciu C, Ranga F, Fetea F, Leopold L, Dulf, F and Parlog R. *Czech J. Food Sc*i, 2009; 27.
11. Liu HX, Sun SQ, Lv GH, Chan KK. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2006; **64**:321-326.
12. http://www.thekitchn.com/smart-tip-keep-berries-fresh-longer-with-this-washing-method-172141: Accessed on, 15th May 2015
13. Yan-qun Li, De-xin Kongd, Hong Wua. *Industrial Crops and Products*, 2013; **41**: 269– 278.
14. Barbara Stuart. Infrared Spectroscopy: Fundamentals and Applications. John Wiley & Sons (UK): 2004.
15. Basri DF, and Fan SH. *Indian J Pharmaco,* 2005; **37:26**-29.
16. Sharma A, Tiwari RK, Kaushik A, Tyagi LK, Shankar K, Virmani T. *Cont J Pharm Sci* , 2007;**1**:9-14.
17. Mishra CK, Pattnaik AK, Rani A, Sasmal D, and Neema RK. *International Journal of Plant Sciences* (Muzaffarnagar), 2009; **4**: 564-568.
18. Dua D, Srivastav NS. *Int J Integr Sci Innov Technol Sec*, 2013; **2:15**-9.