Indian Journal of Traditional Knowledge
Vol 18(3), July 2019, pp 573-578

Karonda and *Jamun* seeds' *in vitro* anticancer efficacy

Vikas Sharma*[,1+], Arti Heer[1], Navneet Kour[1], Akash Sharma[1] & Shashank K Singh[2]

[1]Division of Biochemistry, Faculty of Basic Sciences/Faculty of Agriculture,
Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Main Campus Chatha, Jammu 180 009, J&K, India
[2]Cancer Pharmacology Division, Indian Institute of Integrative Medicine, Canal Road Jammu 180 001, J&K, India

E-mail: [+]vikas.skuast@gmail.com

Received 09 May 2017; revised 05 March 2019

In the search for potential anticancer agents from fruits, the present research work was carried out to examine the *in vitro* cytotoxic potential of seed part of *Carissa carandas* (*karonda*) and *Syzygium cumini* (*jamun*) against nine human cancer cell lines from eight different origins namely MCF-7, T-47D (breast), SF-295 (CNS), HCT-116 (colon), A-549 (lung), MDA-MB-435 (melanoma), OVCAR-5 (ovary), PC-3 (prostate) and A-498 (renal). Methanolic extracts were used as test material and anticancer activity was determined *via* SRB assayat 100 µg/mL. Results revealed that seeds suppressed the proliferation of human cancer cells with growth inhibition range of 78-100% (*karonda*) and 71-93% (*jamun*). *Karonda* seeds exhibited 100% growth inhibition of A-549 and OVCAR-5 cancer cells where as *jamun* seeds displayed 93% growth inhibition of SF-295 cancer cells. The seeds were then evaluated at lower concentrations of 50, 30, 10 and 1 µg/mL in which seeds exhibit significant *in vitro* cytotoxic effect against lung cancer cells (A-549). Further, IC_{50} values were calculated and it was observed that seed extracts from both the fruits showed IC_{50}<10 in case of lung cancer cells whereas *karonda* seed extract also showed IC_{50}<10 in case of colon cancer cells. To conclude, *karonda* and *jamun* seeds possess certain constituents with cytotoxic properties that can be used to develop anticancer agents especially for lung cancer therapy and to provide a great service to cancer patients, further studies are required for the isolation of active ingredients from these seeds.

Keywords: *Karonda*, *Jamun*, Cancer cells, SRB assay, Cytotoxicity

IPC Code: Int. Cl.[19]: A01G 13/00, A01G 13/00, C12Q 1/6886, C12Q 1/6839, A61K 38/00

Cancer is one of the most life threatening diseases, which represents a substantial burden in the community and appears to be a prime cause of concern. Multidisciplinary scientific investigations are making best efforts to combat the disease, but the sure-shot, perfect cure is yet to be brought into the world of medicine. Fruits consumed in our daily diet could be a solution to this deadly disease by providing chemoprotective and chemotherapeutic remedy.

C. carandas is a large dichotomously branched evergreen shrub with short stem and strong thorns in pairs[1], that grows well under tropical and sub tropical climatic conditions[2]. The plant is widely used throughout India in the treatment of scabies, intestinal worms, pruritus and its fruit have been studied for analgesic, anti-inflammatory[3] and lipase[4] activity. The *karonda* fruit is an astringent, antiscorbutic and its root extract is known to have anti-inflammatory and antipyretic properties[5]. Chloroform extract of *karonda* leaves showed good anticancer activity against Caov-3 (ovarian carcinoma) while the n-hexane extract of the unripe fruit showed remarkable activity against lung cancer cells[6,7,8,9].

S. cumini is a widely distributed forest tree in India and other tropical/subtropical regions of the world. Its fruit is a very rich source of anthocyanin and has anti-cancer and anti-viral properties[10,11]. *Jamun* possess antineoplastic[12], radioprotective[13,14,15,16] and chemopreventive effects[17], all of which are useful in the prevention and treatment of cancer[18]. *Jamun* extract has been shown to be selectively cytotoxic to the human neoplastic breast cancer cells and it is logical to suggest that the constituents of *jamun* may have inhibited the process of carcinogenesis by selectively killing the mutated, preneoplastic and neoplastic cells resulting from the carcinogen treatment[19]. The present research work was undertaken to study *in vitro* anticancer potential of seed part of *C. carandas* and *S. cumini* against nine human cancer cell lines (A-498, A-549, HCT-116, MCF-7, MDA-MB-435, OVCAR-5, PC-3, SF-295 and T-47D) from eight different tissues (renal, lung, colon, breast, melanoma, ovarian, prostate and CNS).

*Corresponding author

Methodology

Chemicals

RPMI-1640 medium, dimethyl sulfoxide (DMSO), EDTA, fetal bovine serum (FBS), sulphorhodamine blue (SRB) dye, phosphate buffer saline (PBS), trypsin, gentamycin, penicillin and 5-flurouracil were purchased from Sigma Chemical Co., USA. All other chemicals were of high purity and obtained locally with the brand Sigma-Aldrich Chemicals Pvt. Ltd. and S.D. Fine Chemicals Pvt. Ltd. from Ramesh Traders, Panjthirthi-Jammu, J&K.

Preparation of seed extracts

C. carandas and *S. cumini* were authenticated at site by Dr Vijay Bahadhur Singh, I/c Rainfed Research Station for Subtropical Fruits (RRSSF), Raya, SKUAST-Jammuand enough quantity of fresh fruits were collected.The freshly collected fruits were chopped, seeds were taken out then shade-dried and ground into powdered form. The methanolic extracts of the seeds were prepared by percolating the dried ground plant material (100 g) with 95% methanol and then concentrating it to dryness under reduced pressure. Stock solutions of 20 mg/mL were prepared by dissolving 95% methanolic extract in DMSO. Stock solutions were prepared at least one day in advance and were not filtered. The microbial contamination was controlled by addition of 1% gentamycin in complete growth medium *i.e.*, used for dilution of stock solutions to make working test solutions of 200 µg/mL.

Cell lines/cultures and positive controls

The human cancer cells-A-498 (renal), A-549 (lung), HCT-116 (colon), MCF-7 (breast), MDA-MB-435 (melanoma), OVCAR-5 (ovarian), PC-3 (prostate) SF-295 (CNS) and T-47D (breast) were obtained from National Centre for Cell Science, Pune, India and National Cancer Institute, Frederick, USA. These human cancer cells were further grown and maintained in RPMI-1640 medium. Doxorubicin, 5-Fluorouracil, Mitomycin-C, Paclitaxel and tamoxifen were used as positive controls.

In vitro assay for cytotoxic activity

Extracts were subjected to *in vitro* anticancer activity against various human cancer cell lines[20]. In brief, the cells were grown in tissue culture flasks in growth medium at 37°C in an atmosphere of 5% CO_2 and 90% relative humidity in a CO_2 incubator (Hera Cell, Heraeus; Asheville, NCI, USA). The cells at sub-confluent stage were harvested from the flask by treatment with trypsin (0.05% trypsin in PBS containing 0.02% EDTA) and suspended in growth medium. Cells with more than 97% viability (trypan blue exclusion) were used for determination of cytotoxicity. An aliquot of 100 µL of cells (10^5 cells/mL) was transferred to a well of 96-well tissue culture plate. The cells were allowed to grow for 24 h. Extracts (100 µL/well) were then added to the wells and cells were further allowed to grow for another 48 h.

The anti-proliferative SRB assay which estimates cell number indirectly by staining total cellular protein with the dye SRB was performed to assess growth inhibition. The SRB staining method is simpler, faster and provides better linearity with cell number. It is less sensitive to environmental fluctuations and does not require a time sensitive measurement of initial reaction velocity[21]. In brief, the cell growth was stopped by gently layering 50 µL of 50% (ice cold) trichloroacetic acid on the top of growth medium in all the wells. The plates were incubated at 4°C for 1 h to fix the cells attached to the bottom of the wells. Liquid of all the wells was then gently pipetted out and discarded. The plates were washed five-times with distilled water and air-dried. SRB 100 µL (0.4% in 1% acetic acid) was added to each well and the plates were incubated at room temperature for 30 min.

The unbound SRB was quickly removed by washing the cells five-times with 1% acetic acid. Plates were air-dried, tris buffer (100 µL, 0.01 M, pH 10.5) was added to all the wells to solubilize the dye and then plates were gently stirred for 5 min on a mechanical stirrer. The optical density (OD) was recorded on ELSIA reader at 540 nm. Suitable blanks (growth medium and DMSO) and positive controls (prepared in DMSO and distilled water) were also included. Each test was done in triplicate and the values reported were mean values of three experiments.

The cell growth was determined by subtracting average absorbance value of respective blank from the average absorbance value of experimental set. Percent growth in presence of test material was calculated as under:

- OD Change in presence of control=Mean OD of control–Mean OD of blank
- OD Change in presence of test sample=Mean OD of test sample–Mean OD of blank
- % Growth in presence of control=100/OD change in presence of control

- % Growth in presence of test sample=% Growth in presence of control×OD change in presence of test sample
- % Inhibition by test sample=100–% Growth in presence of test sample

The growth inhibition of 70% or above was considered active while testing extracts, but in testing of active ingredients at different molar concentrations, the growth inhibition of 50% or above was the criteria of activity.

Results and Discussion

The seed extract of *karonda* at 100 µg/mL, showed strong *in vitro* cytotoxic effect against five human cancer cell lines from five different tissues namely HCT-116 (colon), A-549 (lung), MDA-MB-435 (melanoma), OVCAR-5 (ovarian) and A-498 (renal). Maximum growth inhibition *i.e.,* 100% was observed against A-549 and OVCAR-5 cancer cells. The seed showed 89% growth inhibition of HCT-116, 82% of MDA-MB-435 and 78% of A-498 cancer cells. The methanolic extract did not show any cytotoxic effect against other four human cancer cell lines as the growth inhibition against these human cancer cell lines was observed in the range of 63-67%, which is considered in-significant (Table 1). 67% growth inhibition was observed against T-47D (breast), 66% against SF-295 (CNS), 65% against MCF-7 (breast) and 63% against PC-3 (prostate). At lower concentrations, *karonda* showed 95% growth inhibition at 50 µg/mL, 94% growth inhibition at 30 µg/mL and 87% growth inhibitionat 10 µg/mL against A-549 cancer cells. In case of MDA-MB-435, the growth inhibition observed was 79% at 50 µg/mL and 78% at 30 µg/mL whereas the extract also exhibited 86% growth inhibition at 50 µg/mL against HCT-116 cancer cells (Table 2).

Table 1 — Growth inhibitory effect of *karonda* seed along with positive controls against human cancer cells
Growth inhibition of 70% or more in case of extracts has been indicated in bold numbers

Generic name of the fruit	Extract E Extract	Conc. (µg/mL)	Human cancer cell lines from eight different tissues								
			Breast MCF-7	Breast T-47D	CNS SF-295	Colon HCT-116	Lung A-549	Melanoma MDA-MB-435	Ovarian OVCAR- 5	Prostate PC-3	Renal A-498
			Growth Inhibition (%)								
Carissa carandas	Methanolic	100	65	67	66	89	100	82	100	63	78
Positive controls (standard drugs)		Conc. (µM)									
Doxorubicin		1	-	-	71	-	-	-	-	-	-
5-Fluorouracil		20	-	-	-	65	-	-	70	-	-
Mitomycin-C		1	-	-	-	-	-	-	-	63	-
Paclitaxel		1	77	72	-	-	71	-	-	-	70
Tamoxifen		1	-	-	-	-	-	75	-	-	-

Mark (-) indicates that particular human cancer cell line was not treated with that particular positive control

Table 2 — Growth inhibitory effect of *karonda* seed at lower concentrations against human cancer cells
Growth inhibition of 70% or more in case of extracts has been indicated in bold numbers

Generic name of the fruit	Extract E Extract	Conc.(µg/mL)	Human cancer cell lines from five different tissues				
			Colon HCT-116	Lung A-549	Melanoma MDA-MB-435	Ovarian OVCAR-5	Renal A-498
			Growth Inhibition (%)				
Carissa carandas	Methanolic	50	86	95	79	0	39
		30	68	94	78	0	0
		10	61	87	44	0	0
		1	0	09	19	0	0
Positive controls (standard drugs)		Conc.(µM)					
Doxorubicin		1	-	-	-	-	-
5-Fluorouracil		20	65	-	-	70	-
Paclitaxel		1	-	71	-	-	70
Tamoxifen		1	-	-	75	-	-

Growth inhibition of 70% or more in case of extracts has been indicated in bold numbers
Mark (-) indicates that particular human cancer cell line was not treated with that particular positive control
Table 3 Growth inhibitory effect of *jamun* seed along with positive controls against human cancer cells
Growth inhibition of 70% or more in case of extracts has been indicated in bold numbers

Jamun seed extract at 100 µg/mL, exhibited 93% *in vitro* cytotoxic effect against SF-295 cancer cells. It showed 75% growth inhibition of A-498, 74% of HCT-116, 72% of A-549 and PC-3, 71 % of MDA-MB-435 cancer cells. The extract did not exhibit any activity against other three human cancer cell lines as the growth inhibition was found in-significant (Table 3). 65% growth inhibition was observed against OVCAR-5 (ovarian), 61 against T-47D (breast) and 60% against MCF-7 (breast). When tested at lower concentrations, the extract showed 84% growth inhibition at 50 µg/mL and 73% growth inhibition at 30 µg/mL against A-549 cancer cells. It also showed 74% growth inhibition at30 µg/mL against SF-295 cancer cells (Table 4). Further, IC_{50} values were calculated and it was observed that seeds of both the fruits showed IC_{50}< 10 in case of lung cancer cells whereas *karonda* also showed IC_{50}< 10 in case of colon cancer cells (Fig. 1 & Fig. 2).

Cancer is becoming a big load on families and economies. Epidemiological studies have shown that

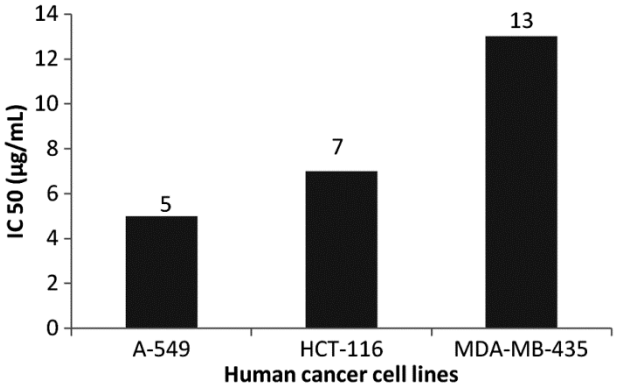

Fig. 1 — IC_{50} values of *karonda* (*Carissa carandas*) seed extract

Table 3 — Growth inhibitory effect of *jamun* seed along with positive controls against human cancer cells
Growth inhibition of 70% or more in case of extracts has been indicated in bold numbers

Generic name of the fruit	Extract E Extract	Conc. (µg/mL)	Human cancer cell lines from eight different tissues								
			Breast MCF-7	Breast T-47D	CNS SF-295	Colon HCT-116	Lung A-549	Melanoma MDA-MB-435	Ovarian OVCAR- 5	Prostate PC-3	Renal A-498
			Growth Inhibition (%)								
Syzygium cumini	Methanolic	100	60	61	93	74	72	71	65	72	75
Positive controls (standard drugs)		Conc. (µM)									
Doxorubicin		1	-	-	71	-	-	-	-	-	-
5-Fluorouracil		20	-	-	-	65	-	-	70	-	-
Mitomycin-C		1	-	-	-	-	-	-	-	63	-
Paclitaxel		1	77	72	-	-	71	-	-	-	70
Tamoxifen		1	-	-	-	-	-	75	-	-	-

Mark (-) indicates that particular human cancer cell line was not treated with that particular positive control

Table 4 — Growth inhibitory effect of *jamun* seed at lower concentrations against human cancer cells
Growth inhibition of 70% or more in case of extracts, has been indicated in bold numbers

Generic name of the fruit	Extract E Extract	Conc. (µg/mL)	Human cancer cell lines from four different tissue					
			CNS SF-295	Colon HCT-116	Lung A-549	Melanoma MDA-MB-435	Prostate PC-3	Renal A-498
			Growth inhibition (%)					
Syzygium cumini	Methanolic	50	0	58	84	50	0	0
		30	74	50	73	21	0	0
		10	6	0	50	31	0	0
		1	6	0	0	54	0	27
Positive controls (standard drugs)		Conc.(µM)						
Doxorubicin		1	71	-	-	-	-	-
5-Fluorouracil		20	-	65	-	-	-	-
Mitomycin-C		1	-	-	-	-	63	-
Paclitaxel		1	-	-	71	-	-	70
Tamoxifen		1	-	-	-	75	-	-

The mark (-) indicates that particular human cancer cell line was not treated with that particular positive control

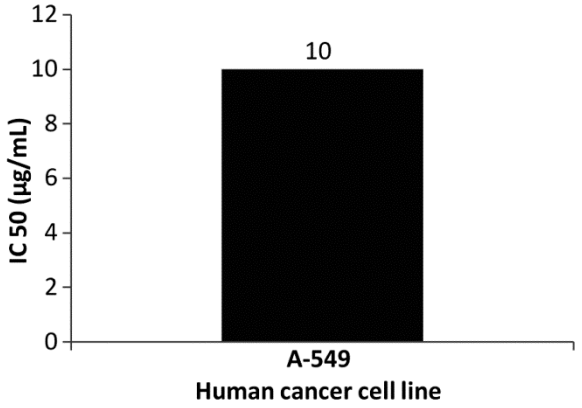

Fig. 2 — IC_{50} values of *Jamun* (*Syzygium cumini*) seed extract

a large number of cancer related deaths could be prevented by increased consumption of fruits. High intake of fruits protect against various cancers and protective effects of high fruit consumption are attributed to vitamins/minerals and phytochemicals, that exhibit a potential for modulating human metabolism in a manner favorable for the prevention of cancer. Therefore, fruits consumed in our daily diet could be a solution to cancer and synergistic effects of phytochemicals in fruits are responsible for their potent antioxidant/anticancer activities. The Jammu region has diversity of subtropical fruits, however, only a relatively small number of fruits have been subjected to accepted scientific evaluation for their potential anticancer effects. Keeping this in mind, the present investigation was carried out to evaluate the *in vitro* anticancer potential of minor fruits from Jammu region against nine human cancer cell lines from eight different tissues. *In vitro* assay for cytotoxic activity was conducted by using SRB dye with appropriate positive controls and the results revealed that methanolic extracts from karonda and jamun showed (cell line specific) *in vitro* cytotoxic activity against human cancer cell lines. The data was compared with literature values and it was found that the data was in agreement with the published data. Extracts of *karonda* were investigated on human ovarian carcinoma, Caov-3 and lung cancer cells[22]. Chloroform extracts of *C. carandas* leaves exhibited cytotoxicity on human ovarian carcinoma cells and n-hexane extract of the unripe fruits is cytotoxic towards the lung cancer cell line[23]. *S. cumini* reduced the tumor incidence, tumor burden and cumulative number of gastric carcinomas[24]. Extract of *jamun* has been shown to be cytotoxic to human neoplastic breast cancer cells and have inhibited the process of carcinogenesis by selectively killing the mutated, preneoplastic and neoplastic cells resulting from the carcinogen treatment[19].

Conclusion

To conclude, *karonda* and *jamun* seeds have shown remarkable activity against lung cancer cells (A-549) at lower concentrations, but karonda also shown significant result in case of melanoma and colon cancer cells. *Karonda* seeds showed IC50< 10 in case of lung and colon cancer cells whereas *jamun* showed IC50=10 against lung cancer cells. In the present research work, an attempt was made to elucidate the *in vitro* anticancer potential of minor fruits from Jammu region. The results obtained from our investigation confirmed the therapeutic potency of *karonda* and *jamun* especially against lung cancer cells. The results also showed that these fruits possess certain cytotoxic constituents that can be used for developing anticancer agents for lung cancer therapy. Moreover, it forms a good basis for the selection of seed part of these fruits for further phytochemical and pharmacological analysis and offer us new drugs from natural sources which would be less toxic and more potent for the efficient management of cancer.

Acknowledgement

The author is thankful to Indian Institute of Integrative Medicine (IIIM) Jammu for providing technical support.

References

1 Wiart C, Medicinal Plants of Asia and the Pacific, *J Nat Prod*, 70, (2006) 251-253

2 Karale AR, Karonda, In: Bose T K, Mitra S K, Sanyal D (Eds.). Fruits-Tropical and Subtropical, Naya Udyog, Kolkata, Vol. II (2002) 721-730

3 Sharma A, Reddy GD, Kaushik A, Shankar K, Tiwari RK, Mukherjee A & Rao CV, Analgesic and anti inflammatory activity of *Carissa carandas* Linn fruits and *Micristylis wallichii* Lindl tubers, *Nat Prod Sci,* 13, (2007) 6-10

4 Mala V & Dahot MU, Lipase activity of *Carissa carandas* fruits, *Science International-Lahore,* 7, (1995)161-164

5 Bhaskar VH & Balakrishnan N, Analgesic, anti-inflammatory and antipyretic activities of *Pergularia daemia* and *Carissa carandas*, *DARU Journal of Pharmaceutical Sciences*, 17, (2009) 168-174

6 Pino J, Marbot R & Vazques C. Volatile flavour constituents of Karonda (*Carissa carandas L.*) fruit, *J Essent Oil Res,* 16, (2004) 432-434

7 Pal R, Kulshreshtha DK & Rastogi RP, A new lignan from *Carissa carandas, Phytochemistry,* 14, (1975) 2302-2303

8 Singh B & Rastogi RP, The structure of carindone, *Phytochemistry,* 11, (1972) 1797-1801

9 Reisch J, Hussain R, Krebs B & Dartmann M, The structure of carissone, *Monatshefte Fuer Chemie,* 121, (1990) 941-944

10 Jain MC & Seshadri TR, Anthocyanins of *Eugenia jambolana* fruits, *Indian J Chem,* 3, (1975) 20-23
11 Venkateswarlu G, On the nature of colouring matter of the jambul fruit (*Eugenia jambolana*), *J Indian Chem Soc,* 29,(1952) 434-437
12 Barh D & Vishwanathan G, *Syzygiumcumin i*nhibits growth and induces apoptosis in cervical cancer cell lines: A primary study, *ecancermedical science,*2, (2008) 83-113
13 Jagetia GC & Baliga MS, *Syzygiumcumini* (jamun) reduces the radiation-induced DNA damage in the cultured human peripheral blood lymphocytes: a preliminary study, *Toxicol Lett,* 132, (2002) 19-25
14 Jagetia GC & Baliga MS. Evaluation of the radioprotective effect of the leaf extract of *Syzygiumcumini* (jamun) in mice exposed to a lethal dose of gamma-irradiation, *Nahrung,* 47, (2003), 181-185
15 Jagetia GC, Baliga MS & Venkatesh P, Influence of seed extract of *Syzygiumcumini* (jamun) on mice exposed to different doses of gamma-radiation, *J Radiat Res,* 46, (2005), 59-65
16 Jagetia GC, Shetty PC & Vidyasagar MS, Treatment of mice with leaf extract of jamun (*Syzygiumcumini*) protects against the radiation-induced damage in the intestinal mucosa of mice exposed to different doses of γ-radiation, *Pharmacology Online,* 1,(2008**)** 169-195
17 Parmar J, Sharma P & Verma P, Chemopreventive action of *Syzygiumcumini* on DMBA-induced skin papillomagenesis in mice, *Asian Pac J Cancer Prev,* 11, (2010) 261-265
18 Sagrawat H, Mann AS & Kharya MD, Pharmacological potential of *Eugenia jambolana*: a review, *Pharmacogn Mag,* 2, (2006) 96-105
19 Li L, Adams LS & Chen S, *Eugenia jambolana* Lam. berry extract inhibits growth and induces apoptosis ofhuman breast cancer but not non-tumorigenic breast cells, *J Agric Food Chem,* 57, (2009) 826-831
20 Monks A, Scudiero D, Skehan P, Shoemaker R, Paull K, Vistica D, Hose C, Langley J, Cronise P, Vaigro-Wolff A, Gray-Goodrich M, Campbell H, Mayo J & Boyd M, Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines, *J Natl Cancer Inst,* 83, (1991) 757-766
21 Skehan P, Storeng R, Scudiero D, Monks A, McMohan J, Vistica D, Warren JT, Bokesch H, Kenney S & Boyd MR, New colorimetric cytotoxicity assay for anticancer-drug screening, *J Natl Cancer Inst,* 82, (1990)1107-1112
22 Sulaiman SF, Teng WS, Leong OK, Yousuf SR & Muhammad ST, Anticancer study of *Carissa carandas* extracts, Project Report, USM, (2008) 1-6
23 Pino, J., Marbot, R. and Vazques, C. 2004. Volatile flavour constituents of Karonda (*Carissa carandas L.*) *fruit. Journal of Essential Oil Research,* **16**: 432-434.
24 Goyal PK, Verma P& Sharma P, Evaluation of anti-cancer and anti-oxidative potential of *Syzygium cumini* against benzo[a]pyrene (BaP) induced gastric carcinogenesisin mice, *Asian Pac J Cancer Prev,* 11**,** (2010)753-758