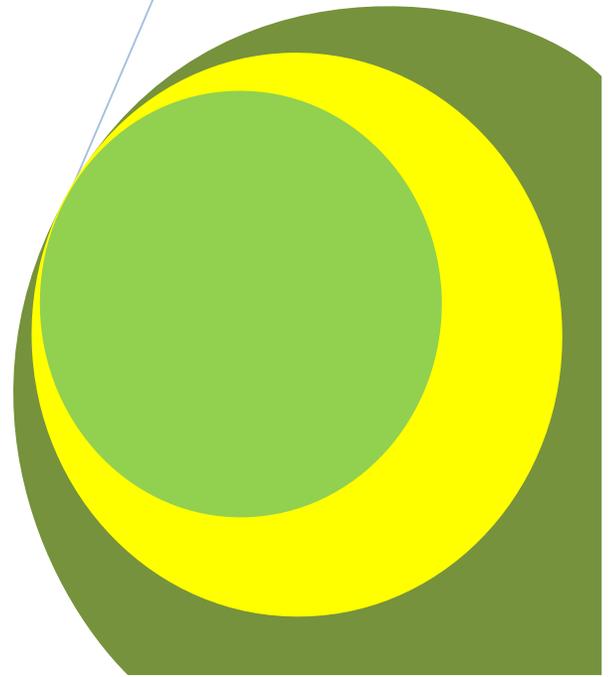


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Anticancer Activity of *Carica papaya* Extracts in *vito* and Phytochemical Analysis

By

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Research Article

Anticancer Activity of *Carica papaya* Extracts *in vitro* and Phytochemical Analysis

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ABSTRACT

The aim of the present work is the evaluation of anti cancer effect of *Carica papaya* aerial parts extracts and also the investigation of the phytoconstituents from the extracts of the plant. Petroleum ether (40-60°C), Chloroform, ethyl acetate and methanol 80% extracts of *C. papaya* aerial parts were tested for their anti cancer activity on three cancer cells TK10 (renal), UACC62 (melanoma) and MCF7 (breast) cancer cells using a Sulforhodamine B (SRB) assay. Petroleum ether of *C. papaya* at the concentration of 100 µg/ml has shown a significant anticancer effect for MCF7 (breast) cancer cells and showed less anticancer effect for the other two cancer cells while the other extracts have mild anticancer effect on the three cancer cells. Phytochemical profile of the plant extracts proves the presence of flavonoids, tannins, alkaloids, carbohydrates and triterpenes and bioactive fractionation of petroleum ether extract revealed the isolation and identification of β-sitosterol, stigmasterol as the major constituents. The results suggest that petroleum ether extract of *C. papaya* exhibited a significant anti cancer activity for breast cancer.

Keywords: *Carica papaya*, aerial parts, anticancer activity, phytoconstituents.

INTRODUCTION

Cancer is a leading cause of death worldwide, it is a dreadful disease, and combating this disease is of great importance to public health. There is a necessity for search of new natural extracts of plant source and compounds with cytotoxic activity as the treatment of cancer with the available anticancer drugs is often unsatisfactory due to the problem cytotoxicity to the normal cells. Recent Phytochemical examination of plants which have a suitable history of use in folklore for the treatment of cancer has often resulted in the isolation of principles with anti cancer activity (Afolabi *et al.*, 2007). Phytochemical examination has been making rapid progress and herbal products are becoming popular as sources of plausible anticancer compounds (Parag *et al.*, 2010). In our searching for new anticancer drugs from plants, *Carica papaya* Linn. from Caricaceae family known as pawpaw tree is a vegetable fruit widely distributed throughout the world, mostly grow in tropics and it grows up to 5 to 10 m tall (Duke, 1984). The ripe fruit is edible and is usually eaten raw, without the skin or seeds. The unripe green fruit (which is a rich source of vitamin A) can be eaten cooked, usually in curries, salads and stews as used in Thai cuisine (Lohiya *et al.*, 2002). The papaya fruit, as well as all other parts of the plant, contain a milky juice in which an active principle known as papain is present (Nisar *et al.*, 2011). It has a good effect as a remedy in dyspepsia and kindred ailments. The juice has been in use on meat to make it tender (Nisar *et al.*, 2011). The seed is used for intestinal worms when chewed. The root is chewed and the juice swallowed for cough, bronchitis, and other respiratory diseases. The unripe fruit is used as a remedy for ulcer and impotence (Aiyeloja and Bello, 2006). Previous studies on biological activities of *C. papaya* parts, extracts and isolated compounds showed that the latex and root extracts inhibited *Candida albicans* while extracts of pulp and seeds showed bacteriostatic properties against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*, and *Entamoeba histolytica*, *in vitro* (Emeruwa, 1982). Its root aqueous extract has equally been shown to have purgative effect (Akah *et al.*, 1997). In another study, the hypoglycemic and hypolipidemic effects of the aqueous seed extract of *C. papaya* was reported and the LD₅₀ of the oral toxicity was estimated to greater than 2000 mg/kg/oral route (Adeneye and Olagunju, 2009). This study was carried out to evaluate anti cancer effect of *C. papaya* extracts on three cancer cells TK10 (renal), UACC62 (melanoma) and MCF7 (breast) cancer cells and also to investigate the phytoconstituents of the plant extracts.

MATERIALS AND METHODS

Experimental

Spectroscopic data: NMR–Varian. MS (Finnigan MAT SSQ 7000, 70 ev). Silica gel (60-200 mesh, Merck). Thin Layer Chromatography (TLC): pre-coated sheets of silica gel 60 F₂₅₄ (Merck). Sephadex LH-20 (Sigma).

Plant Material

The aerial parts of *C. papaya* were collected from Al-Zohiriya garden, Giza, Egypt in May 2011. The plant was identified by Dr. Mohammed El-Gebaly, Department of Botany, National Research Centre (NRC) and by Mrs. Tereza Labib Consultant of Plant Taxonomy at the Ministry of Agriculture and director of Orman botanical garden, Giza, Egypt. A voucher specimen is deposited in the herbarium of Al-Zohiriya garden, Giza, Egypt.

Preparation of the extracts

Finely ground aerial parts from *C. papaya* 700 g were extracted with petroleum ether (40-60°C) chloroform, ethyl acetate and methanol 80% solvents by maceration. Each extract was concentrated to dryness to yield, 9 g of petroleum ether, 6.7 g of chloroform, 5.5 g of ethyl acetate and 37 g of methanol 80% extracts, respectively. Each extract was tested for the presence of the phytoconstituents according to the following standard tests, Molisch's test for carbohydrates, Shinoda test for flavonoids, forth test for saponins, Salkowski 's for terpenes and sterols, FeCl₃ and Mayer's reagents for detecting of tannins and alkaloids, respectively (Sofowra 1993, Trease and Evans 1989, Harborne 1973).

Phytochemical characterization of Petroleum ether extract

Petroleum ether extract 7 g was subjected to silica gel column chromatography using petroleum ether as eluent and gradually increasing amount of ethyl acetate (EtOAc) where two major compounds were isolated. Compound 1 (β -sitosterol) was isolated from CHCl₃: EtOAc (90:10) elution and compound 2 (stigmasterol) was isolated through elution with petroleum ether: EtOAc (95:15).

Sulforhodamine B (SRB) assay

The growth inhibitory effects of the extracts were tested in the 3-cell line panel consisting of TK10 (renal), UACC62 (melanoma) and MCF7 (breast) cancer cells using a Sulforhodamine B (SRB) assay. The SRB assay was developed by (Skehan *et al.*, 1990) to measure drug-induced cytotoxicity and cell proliferation. Its principle is based on the ability of the protein dye sulforhodamine B (Acid Red 52) to bind electrostatically in a pH-dependent manner to protein basic amino acid residues of trichloroacetic acid-fixed cells. Under mild acidic conditions, it binds to the fixed cellular protein, while under mild basic conditions it can be extracted from cells and solubilized for measurement. The SRB assay is performed at CSIR in accordance with the protocol of the Drug Evaluation Branch, NCI (National Cancer Institute). The 3-cell line panel used is recommended by the NCI for preliminary screens. The human cell lines TK10, UACC62 and MCF7 were previously obtained from NCI in the framework of a collaborative research program between CSIR and NCI. Cell lines were routinely maintained as monolayer cell cultures in RPMI containing 5% fetal bovine serum, 2 mM L-glutamine and 50 μ g/ml gentamicin. For the screening experiment, the cells (3-19 passages) were inoculated in 96-well microtiter plates at plating densities of 7-10 000 cells/well and were incubated for 24 h. After 24 h, one plate was fixed with TCA to represent a measurement of the cell population for each cell line at the time of drug addition (T₀). The other plates with cells were treated with the experimental samples which were previously dissolved in DMSO as 10000 μ g/ml stocks and diluted in medium to a final concentration 100 μ g/ml. Cells without samples served as controls. Blank wells contained complete medium without cells. Emetine was used as a reference standard. The plates were incubated for 48 h after addition of the extracts (100 μ g/ml). At the end of the incubation period, the cells were fixed to the bottom of each well with cold 50% trichloroacetic acid, washed, dried and dyed with SRB. Unbound dye was removed and protein-bound dye was extracted with 10mM Tris base for optical density determination at a wavelength 540 nm using a multiwell spectrophotometer. Optical density measurements were used to calculate the net percentage cell growth. The optical density of the test wells after 48-h period of exposure to test compound is T_i, the optical density at time zero is T₀, and the control (untreated cells) optical density is C. Percentage cell growth is calculated as: $[(T_i - T_0)/(C - T_0)] \times 100$ for concentrations at which T_i \geq T₀ $[(T_i - T_0)/T_0] \times 100$ for concentrations at which T_i < T₀.

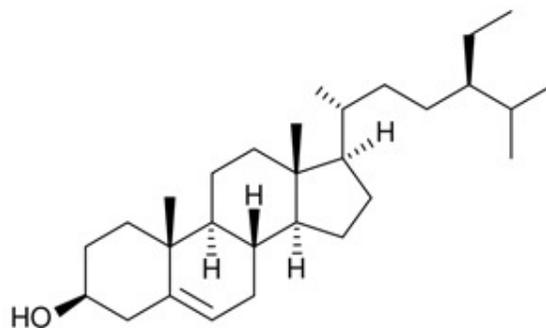
RESULTS AND DISCUSSION

Phytochemical analysis of *C. papaya* extracts is shown in table 1, it showed that petroleum ether extract has triterpenes and/or sterols, chloroform extract has alkaloids and triterpenes, ethyl acetate extract has flavonoids and tannins while methanol 80% extract has flavonoids, tannins, alkaloids, carbohydrates and triterpenes. The isolated compounds from petroleum ether extract of *C. papaya* aerial parts are shown in Fig. 1.

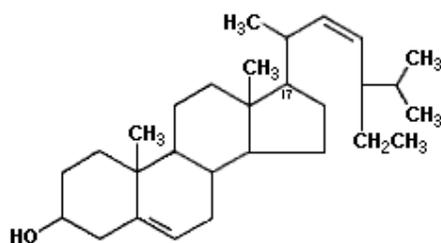
Table 1: Phytochemical Analysis from the *C. papaya* aerial parts extracts

Constituents	Petroleum ether	Chloroform	Ethyl acetate	Methanol 80%
Triterpenes and /or Sterols	+	+	-	+
Carbohydrates and/or glycosides	-	-	-	+
Flavonoids	-	-	+	+
Coumarins	-	-	-	-
Alkaloids and/or nitrogenous compounds	-	+	-	+
Tannins	-	-	+	+
Saponins	-	-	-	-

(+) presence of constituents, (-) absence of constituents



Compound 1: β -sitosterol



Compound 2: Stigmasterol

Fig. 1: Compounds isolated from petroleum ether extract of *C. papaya* aerial parts

Structure elucidation of the bioactive compounds isolated of Petroleum ether extract of *C. papaya*

β -sitosterol (1): 20 mg, white needles, $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ 5.37 (1H, m, H-6), 3.52 (1H, m, H-3), 1.09 (3H, s, CH_3 -19), 0.98 (3H, d, $J=6.5$, CH_3 -21), 0.92 (3H, t, $J=7.4$, CH_3 -29), 0.85 (3H, d, $J=6.7\text{Hz}$, CH_3 -26), 0.81 (3H, d, $J=6.7\text{Hz}$, CH_3 -27), 0.75 (3H, s, CH_3 -18). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3): δ 140.4 (C-5), 121.5 (C-6), 71.6 (C-3), 57.2 (C-17), 56.4 (C-14), 50.3 (C-9), 46.3 (C-24), 42.8 (C-13, 4), 39.8 (C-12), 37.6 (C-1), 36.7 (C-10), 35.9 (C-20), 34.2 (C-22), 31.7 (C-8, 7), 31.4 (C-2), 29.2 (C-25), 28.4 (C-16), 26.2 (C-23), 24.5 (C-15), 23.4 (C-28), 21.1 (C-11), 19.8 (C-26), 19.5 (C-19), 19.2 (C-27), 18.6 (C-21).

Stigmasterol (2): 17 mg, white needle crystals, $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ 5.32 (1H, m, H-6), 5.11 (1H, dd, $J=14.2, 8.2$ Hz, H-22), 5.04 (1H, dd, $J=14.2, 8.2$ Hz, H-23), 3.54 (1H, m, H-3), 1.04 (3H, s, CH_3 -10), 0.9 (3H, d, $J=6.5$, CH_3 -20), 0.84 (3H, d, $J=7.4$, CH_3 -27), 0.82 (3H, d, $J=7.4$, CH_3 -26), 0.68 (3H, s, CH_3 -13). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3): δ 140.6 (C-5), 138.4 (C-22), 129.1 (C-23), 121.8 (C-6), 71.9 (C-3), 56.7 (C-17), 56.9 (C-14), 50.9 (C-9), 50.7 (C-24), 42.6 (C-13, 4), 39.6 (C-12), 37.4 (C-1), 40.2 (C-20), 36.7 (C-10), 31.4 (C-8, 7), 31.7 (C-2), 30.9 (C-25), 28.8 (C-16), 24.8 (C-15), 24.7 (C-28), 21.5 (C-11), 20.8 (C-26), 20.4 (C-19), 19.7 (C-27), 19.1 (C-21).

Chromatographic separation and purification of the petroleum ether resulted in isolation and identification of compound 1 (β -sitosterol) which gave dark spot under short UV light that changed to violet colour on spraying with vanillin sulphuric and heating in an oven at 110°C for 5min and also compound 2 (stigmasterol) gave a dark spot under short UV light and also gave a violet colour after spraying with vanillin sulphuric and heating in an oven at 110°C for 5min. NMR spectral data has shown signals very close to compound 1 (β -sitosterol), also it is identified by other authors (Pateh et al. 2009). Similarly, a comparison of the NMR spectral data of compound 2 (Pateh et al. 2009; Shirin et al., 2012) with published data allowed the identification of compound 2 as stigmasterol; confirmation of both compounds was done by co-TLC with authentic standards. The extracts of *C. papaya* aerial parts were tested for their anticancer activities on different cancer cells, TK10 (renal), UACC62 (melanoma) and MCF7 (breast) cancer cells using Sulforhodamine B (SRB) assay (Skehan et al., 1990). Petroleum ether ($40\text{-}60^\circ\text{C}$), Chloroform, ethyl acetate and methanol 80% extracts of *C. papaya* aerial parts have a mild anticancer effect for TK10 (renal) and UACC62 (melanoma) cancer cells while petroleum ether has a significant anticancer effect for MCF7 (breast) cancer cells with comparison with a standard drug (Emetine) and the other extracts showed a mild anticancer effect for this cancer cell (table 2). The significant anticancer effect of petroleum ether extract of *C. papaya* is may be due the presence of the bioactive isolated compounds, β -sitosterol and stigmasterol from the extract. In previous study, *Chorisia crispiflora* n-hexane extract has a significant effect on growth inhibition and induction of apoptotic processes in MCF-7 breast cancer cells and the activity is due to the presence of β -sitosterol, β -sitosterol 3-glucoside and stigmasterol 3-glucoside (Samar et al., 2013) and in another reported study that β -sitosterol increased the activities of antioxidant enzymes, superoxide dismutase and glutathione peroxidase in cultured macrophage cells with oxidative stress induced by phorbol 12-myristate 13-acetate, indicating that phytosterols can protect cells from damage by reactive oxygen species (Vivancos and Moreno, 2005).

Table 2: Anti cancer effect of *C. papaya* aerial parts extracts

Extracts	Con., $\mu\text{g/ml}$	Growth TK10, %	SD	Growth UACC62, %	SD	Growth MCF7, %	SD
Petroleum ether extract	100	-25.54	0.037	-42.17	0.059	-62.91	0.080
Chloroform	100	88.10	0.019	48.08	0.042	53.89	0.091
Ethyl acetate	100	74.23	0.012	40.89	0.016	33.68	0.020
Methanol 80%	100	-18.69	0.001	-19.32	0.071	-29.06	0.021
EMETINE	10μM	-61.35	0.007	-86.66	0.006	-46.41	-61.35

SD is standard deviation. % Growth is the net growth of the cells in treated wells compared to untreated controls over the 48h experimental period, i.e. 100% growth means there are the same amount of cells in treated wells as in untreated control wells; 0% growth means the treated wells contain the same number of cells as at the start of the incubation, time 0 (thus no increase in cell number); -100% growth means there are no cells left in the well after 48h. Emetine as a standard.

CONCLUSION

The reported results show that petroleum extract of *C. papaya* aerial parts has significant anticancer effect on MCF7 (breast) cancer cells. Thus, *C. papaya* aerial parts could be helpful in cancer prevention and treatment. *C. papaya* could be a natural source of anticancer compounds with anti proliferative and/or apoptotic properties and as well, due to its anticancer pharmacological effect, clinical trials are recommended to evaluate the beneficial effects of this plant in human models.

Conflict of interest

There is no conflict of interest associated with the authors of this paper.

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