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Anticarcinogenic Properties of *Strobilanthes crispus* Extracts and its Compounds *in vitro*

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Abstract: The antiproliferative activities of *Strobilanthes crispus* was evaluated using (3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazoliumbromide) (MTT) assay against several cancer cell lines and Chang liver (normal) cell lines. The methanolic extracts of *Strobilanthes crispus* displayed the strongest cytotoxic effect on colon cancer (Caco-2) followed by human breast cancer hormone non-dependent (MDA-MB-231) and liver cancer (HepG-2) with IC₅₀ values of 22.3, 27.2 and 29.3 µg mL⁻¹, respectively. The chloroform extract of this plant was shown to also have cytotoxic effect against Caco-2 and HepG-2 with IC₅₀ values of 25.1 and 28.0 µg mL⁻¹, respectively. β-sitosterol and stigmasterol were isolated from the leaves of *Strobilanthes crispus*. β-sitosterol displayed cytotoxic properties against Caco-2, HepG-2 and MCF-7 with IC₅₀ values of 20.0, 53.0 and 71.2 µM, respectively. Whereas, stigmasterol inhibited the proliferation of Caco2, HepG2, MCF-7 and MDA-MB-231 with IC₅₀ values of 132.5, 182.5, 156.0 and 185.9 µM, respectively. There was no cytotoxic effect observed on normal cell lines (Chang liver) in all samples tested.

Key words: *Strobilanthes crispus*, anticarcinogenic properties, MTT assay

Plant Material

Strobilanthes crispus (Acanthaceae) was harvested from the herbal garden in Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Malaysia. The herbarium voucher specimen was identified and deposited by Mr. Ahmed Zainuddin from Department of Botany, Faculty of Science and Technology, Universiti Kebangsaan Malaysia. The voucher number for *Strobilanthes crispus* was AZ-6803.

Uses in Traditional Medicine

Strobilanthes crispus has been used as antidiabetic, antilytic, laxative (Sunarto, 1977), anti-AIDS, anti-leukimic (Kusumoto *et al.*, 1992). The leaves of *Strobilanthes crispus* possessed high antioxidant activity (Ismail *et al.*, 2000) and anti-hepatocarcinogenesis (Jaksa *et al.*, 2004).

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Previously Isolated Classes of Constituents

The compounds that were isolated from *Strobilanthes crispus* were ester glycosidic compounds of caffeic acid (verbascoside) (Soediro *et al.*, 1983), 7 phenolic acid (p-hydroxy benzoic, p-voumeric, caffeic, vanilic, gentinic, ferulic and syringic (Soediro *et al.*, 1987), β -sitosterol and stigmasterol.

Tested Material

The leaves were dried in the oven at 60°C overnight and were grounded to fine powder. Three types of methods were applied for extraction. The first was catechin extraction (Hara, 1994). The second was single solvent extraction using ethanol, methanol and chloroform (Ali *et al.*, 1996). The third was stepwise extraction using solvents of increasing polarity. The solvents used were hexane, chloroform, ethyl acetate and methanol. Bioactive compounds (β -sitosterol and stigmasterol) were isolated from leaves of *Strobilanthes crispus* which were provided by Assoc. Prof. Dr. Taufik Yap-Yun Hin.

Studied Activity

In vitro cytotoxicity of plant extracts and bioactive compounds were determined using 3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide (MTT) (Roche, USA) assay.

Cell Lines

Liver cancer (HepG-2), breast cancer hormone dependent (MCF-7), breast cancer hormone non-dependent (MDA-MB-231), colon cancer (Caco-2) and normal liver cell lines (Chang liver) were obtained from the American Type Culture Collection (ATCC), Rockvilles, Maryland, USA. The medium for HepG-2 and Chang liver were Minimum Essential Medium with Earle's salt (Gibco, USA) while Caco-2, MDA-MB-321 and MCF-7 were grown in Dulbecco's modified Eagle medium (Gibco, USA). The cells were cultured in medium supplemented with 10% fetal calf serum, 100 IU⁻¹ penicillin and 100 μ g mL⁻¹ of streptomycin (Gibco, USA) using 25 cm² flasks (Nunc, Denmark), in a 5% CO₂ incubator (Sanyo, Japan) at 37°C.

Results

Table 1 showed that the methanolic extracts of *Strobilanthes crispus* displayed cytotoxic effect on Caco-2 followed by MDA-MB-231 and HepG-2 with IC₅₀ values of 22.3, 27.2 and 29.3 μ g mL⁻¹, respectively. The chloroform extract possessed cytotoxic effect against Caco-2 and HepG-2 with IC₅₀ values of 25.1 and 28.0 μ g mL⁻¹, respectively (Table 2). Table 3, β -sitosterol displayed antiproliferative properties against Caco-2, HepG-2 and MCF-7 with IC₅₀ values of 20.0, 53.0 and 71.2 μ M, respectively. Whereas, stigmasterol inhibited the proliferation of Caco-2, HepG-2, MCF-7 and MDA-MB-231 with IC₅₀ values of 132.5, 182.5, 156.0 and 185.9 μ M, respectively. There was no cytotoxic effect observed on normal cell lines (Chang liver) in all samples tested.

The IC₅₀ (concentration that inhibit 50% of cell proliferation) values were obtained from methanol extract and chloroform extract (based on the polarity) of *Strobilanthes crispus*. The bioactive compounds (β -sitosterol and stigmasterol) displayed strong cytotoxic effects on selected cancer cell lines. Both the crude extracts and bioactive compounds showed great potential

Table 1: IC₅₀ (µg mL⁻¹) values of *Strobilanthes crispus* catechin and solvent extraction against several cancer cell lines and chang liver cell lines

Extracts	HepG-2	Caco-2	MCF-7	MDA-MB-231	Chang liver
Catechin	>100	>100	>100	>100	>100
Ethanol	>100	>100	>100	>100	>100
Methanol	29.3	22.3	>100	27.2	>100
Chloroform	>100	>100	>100	>100	>100

Table 2: IC₅₀ (µg mL⁻¹) value of *Strobilanthes crispus* (extraction with solvent of increasing polarity) against several cancer cell lines and chang liver cell lines

Extracts	HepG-2	Caco-2	MCF-7	MDA-MB-231	Chang liver
Hexane	>100	>100	>100	>100	>100
Chloroform	28	25.1	>100	>100	>100
Ethyl acetate	>100	>100	>100	>100	>100
Methanol	>100	>100	>100	>100	>100

Table 3: IC₅₀ (µM) value of β-sitosterol and stigmasterol against several cancer cell lines and chang liver cell lines

Compound	HepG-2	Caco-2	MCF-7	MDA-MB-231	Chang liver
β-sitosterol	53.0	20.0	71.2	>247.5	>247.5
Stigmasterol	182.0	132.5	156.0	185.9	>242.7

as chemotherapeutic agents. At the range of concentration tested, no cytotoxic activity was observed in normal Chang liver cell lines treated with the above extracts and compounds.

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