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Review Article

Medicinal Properties of *Strobilanthes crispus*: A Review

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Introduction

In spite of the breakthrough innovation in combinatorial chemistry and molecular modelling, natural products, especially medicinal plants remain one of the important sources from which pharmacologically active compounds are isolated, tested and evaluated in the discovery of new drugs, new leads and chemical entities in pharmaceutical development [1-3]. In fact, many of the prescription drugs derived from plants have been approved for the clinical use in treating cancer, malaria and other metabolic diseases [3]. For example, paclitaxel used in the treatment of various cancers is derived from the bark of the Pacific yew tree, *Taxus brevifolia* [4]. Vinblastine isolated from Madagascar periwinkle plant (*Vinca rosea*) is also useful in treating cancers such as leukaemia, testicular teratoma and Hodgkin's disease [5]. Natural products have recently regained its prominent role in drug discovery with the increasing recognition of significance of their structural diversity and expanding exploration of their therapeutic use.

One of the plants that have elicited a great deal of interests and attention among researchers of late is *Strobilanthes crispus* (L.) Blume, which is a woody shrub found distributed throughout the regions of Madagascar to Indonesia [6]. It is locally identified by other common names such as "daun picah beling" in Jakarta or "enyoh kelo", "kecibeling", or "bejibeling" in Java [6] and as "pecah kaca" or "jin batu" in Malaysia. A mature plant usually reaches a height of 1 to 2 m and can be found growing wild along the river banks, in abandoned fields or cultivated. The leaves of *S. crispus* are described as oblong-lanceolate, rather obtuse, with the edge shallowly crenated and covered with short hairs on both surface [6]. The upper surface of the leaves is in a shade of darker green and less rough when compared to the underside. The yellow-coloured flowers of the plant are short, dense and are panicled spikes.

The leaves of the plant are the part used in folklore medicine in Malaysia and Indonesia. Traditionally, fresh or dried leaves were boiled with water and the infusion made has been shown to have anti-diabetes, anticancer, laxative, diuretic and antilytic properties [6, 7]. The dried form may have a longer shelf life preserved in a sealed bag, away from sunlight, heat and moisture. Topical application of macerated leaves on wounds caused by poisonous snakebites was reported to have toxin neutralization effect along with pain and swelling alleviation. Fresh leaves are masticated and swallowed to improve the immune system as indicated by a survey carried out among the indigenous people living in Perak of West Malaysia [8]. The plant has been promoted as containing a rich source of cystoliths of calcium carbonate and the infusion is slightly alkaline [7] thus aid in urination [9]. Daily tea consumption of *S. cripus* contains catechin, serving as potential antioxidant in cancer prevention [10].

In recent years, herbal preparations of *S. crispus* are increasingly used by the general public as an alternative option to promote overall well-being as well as for therapeutic and disease preventing purposes. The synergistic and side effects reducing properties of the plant when used with current treatment have widely been purported in local community. However, the mechanism of action, potency, efficacy and safety is still poorly understood and studied. Hence, additional research is required to be carried out to establish a strong scientific basis for its promoted use before the large-scale commercialisation. In this review, we will focus on discussing the scientifically proven pharmacological properties of the plant including anti-carcinogenesis, anti-glycaemia, antioxidant and wound healing; together with its mode of action and the future potential to be used as therapeutic drug.

Anti-Carcinogenic Properties

Several studies have reported the potential of *S. crispus* as an anti-cancer agent. In previous studies, *S. crispus* extracts demonstrated inhibitory property against breast [11-16], liver [11, 14, 17-19], prostate [15] and colon [11] carcinomas. Different extraction solvents were used in the studies. The ethanol extract of *S. crispus* has been shown to reduce cell viability and proliferation in hormone-dependent breast adenocarcinoma (MCF-7) as detected by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and BrdU (5-bromo-2'-deoxyuridine) assays [16]. The cytotoxic effect was better in MCF-7 with relatively lower value of half maximal inhibitory concentration (IC_{50}) (30 µg/mL) than

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in hormone-independent breast cancer cells (MDA-MB-231) (IC_{50} > 100 µg/mL). In other studies, the anti-proliferative effects of methanolic and chloroform extracts were investigated against a few cancer cell lines as well as Chang liver cells. Both the crude extracts were not cytotoxic against Chang liver cell line. Meanwhile, methanolic extract showed considerable cytotoxicity against colon cancer (Caco-2), with IC_{50} value of 22.3 µg/mL, followed by MDA-MB-231 (IC_{50} 27.2 µg/mL) and lastly hepatocarcinoma cells (HepG-2) (IC_{50} 29.3 µg/mL). The chloroform extract was cytotoxic against Caco-2 and HepG-2 with IC_{50} values of 25.1 and 28.0 µg/mL respectively [11]. Among the cancer cell lines tested, Caco-2 was most sensitive towards cytotoxic effect of *S. crispus* with lowest IC_{50} value. In terms of solvents used, methanol displayed greater potential than others, considering the responsiveness of various cancer cell types towards its treatment.

Besides crude extracts, fractionated compounds from *S. crispus* was also used in previous research. Selected subfractions of dichloromethane (DCM) extract of *S. crispus* were evaluated for its cell death induction property in breast and prostate cancer (PC-3 and DU-145) cell lines [15]. From the results, SC/D-F9 fraction showed statistically significant cytotoxicity in MCF-7, MDA-MB-231, PC-3 and DU-145 cells with relatively low effective concentration (EC_{50}) values while being non-toxic to the normal breast epithelial cell line. The EC_{50} values for the four cancer cell lines were 8.5, 10.0, 7.4 and 7.2 µg/mL respectively. SC/D-F9 fraction demonstrated better cell killing activity in comparison to some of the chemotherapeutic drugs. SC/D-F9 increased the percentage of cell death from 44% to 57% in MCF-7 within 48 hours whilst cell death induced by tamoxifen declined by almost 20% for the same time period. The decrease in cell death over time is possibly associated with the emergence of cell resistance to tamoxifen. Other chemotherapeutic drugs such as paclitaxel and doxorubicin also showed poor activity with low cytotoxicity of 24% and 9% respectively. In PC-3 cells, the percentage of cell death tripled to almost 90% after SC/D-F9 treatment for 48 hours; and this effect is comparatively higher than the effect seen after treatments with docetaxel, paclitaxel and doxorubicin. It can be inferred that SC/D-F9 was cytotoxic to both prostate and breast cancer cell lines with different efficacies, and its effect was better than some commercialized anti-cancer drugs. This study shows that evaluation of cytotoxic effects of a compound on different cell lines of a cancer type was necessary as the different cell lines may display different sensitivities towards the anticancer compound.

Further purification of compounds from *S. crispus* extract has led to the discovery of 2 potential active compounds: ß-sitosterol and stigmasterol. The cytotoxicity effect of ß-sitosterol was found particularly in Caco-2, HepG-2 and MCF-7 cells with IC_{50} values of 20.0, 53.0 and 71.2 µM, respectively whereas stigmasterol lowered the viability of Caco-2, HepG-2, MCF-7 and MDA-MB-231 cells with IC_{50} values of 132.5, 182.5, 156.0 and 185.9 µM, respectively [11]. In addition, ß-sitosterol treatment was reported to decrease cell viability in leukemia [20], prostate cancer [21], breast cancer [22] and fibrosarcoma [23] while stigmasterol reduced ovarian cancer risk [24].

A variety of mechanisms has been proposed to explain the chemopreventive effect of *S. cripus*. Induction of apoptosis in which tumour cell undergoes self-destruction via caspases-dependent pathway was widely proposed as the mechanism of action of *S. crispus*. The two mechanisms involved in apoptosis are extrinsic (stimulation of death receptor) and intrinsic (mitochondria-mediated) pathways, both of which reaching a convergent point at the executioner phase. Extrinsic pathway is mediated via the interaction between death receptor Fas and its ligand FasL. Formation of death-inducing-signalling-complex (DISC) subsequently activates initiator caspases 8 by auto-catalysis, which in turn causes proteolytic cleavage of effector capases 3/7 [25]. The intrinsic pathway, however, is dependent on the balance between the pro- and anti-apoptotic proteins of Bcl-2 family which regulates the permeability of mitochondrial membrane [26]. Disruption of this balance may lead to the activation of pro-apoptotic protein Bax with subsequent mitochondrial translocation and oligomerisation [27] to form the permeability transition (MPT) pore through which apoptogenic factors such as cytochrome C and apoptosis protease activating factor 1 (Apaf-1) are diffused out from the intermembrane space [28, 29]. Once in the cytoplasm, cytochrome C combines with Apaf-1 to form apoptosome, which activates caspase 9, a key initiator factor of intrinsic pathway [30]. Activation of caspases 3/7 by caspases 9 triggers a chain of proteolytic action leading to cell shrinkage, pyknosis, karyorrhexis, and membrane blebbing [31].

S. crispus induced apoptosis in cells was evidenced when Chong et al. (2012) showed that after treatment with *S. crispus* ethanolic extract at IC_{50}, MCF-7 cells displayed characteristic apoptotic features [16]. Flow cytometry cell cycle analysis and Tunnel assay revealed approximately 35 and 47% of $subG_1$ peaks and 30 and 50% of Tunnel positive cells when the cells were treated for 48 and 72 hours respectively. The increased DNA fragmentation and hypo-diploid subpopulation indicates that the cytotoxicity of *S. crispus* was induced via apoptosis. The mechanism involved is intrinsic/mitochondrial activated apoptotic pathway as the concentration of cytochrome C increased significantly from 24 to 36 hours in *S. crispus*- treated MCF cells. This is correlated with the mitochondrial permeability which was markedly reduced in membrane potential as observed in another study carried out recently [32]. The resulting increase in the activity of caspases 3/7 with a fold change of nearly 3 was also observed along with an induction of p53 expression and X-linked inhibitor of apoptotic protease (XIAP) suppression [16]. Interestingly, p53 (tumour suppressor gene) may also promote apoptosis by transcription dependent and independent mechanisms. Binding to the p53 responsive element causes up-

regulation of a small set of pro-apoptotic proteins (Bax) [33] as well as down-regulation of anti-apoptotic genes (Bcl2, BclXL and Survivin). XIAP, however, prevents apoptosis by suppressing the activity of capases 9.

The ß-sitosterols present in *S. crispus* have been suggested to promote apoptosis pathways through down-regulation of Bcl-2 proteins [20] and increased activity of pro-apoptosis enzyme [23], resulting in activation of caspases-3 activity [20] mediated by external stimuli Fas pathway and internal stimuli Bax [34].

Another mechanism of action of *S. crispus* is by down-regulation of oncogene, c-Myc. *S. crispus* was found to inhibit the c-Myc expression in HepG-2 cells [12]. The proto-oncogene c-Myc is a transcription factor involved in regulating most cellular functions, including proliferation, growth, metabolism, differentiation, genome stability and apoptosis [35]. Oncogene c-Myc is activated by enchanced transcription [36, 37], chromosomal rearrangement [38, 39] and resistance to ubiquitin-mediated degradation [40, 41]. c-Myc is overexpressed in majority of malignancies, including breast, prostate, colorectal, hepatocellular carcinoma, lymphoma, melanoma, and myeloid leukemia [42]. Downregulation of c-Myc expression by interference approach (antisense and short interfering RNAs) was found to induce apoptosis in melanoma [43], breast cancer [44, 45] and prostate cancer [46, 47].

Scientists also evaluated the mechanism of action of *S. crispus* through angiogenesis. Angiogenesis is stimulated by tumour cells to support their growth and metastasis. Inhibition of angiogenesis induces apoptosis by depriving the tumour of essential nutrient and oxygen supply, making it a potential target exploited for cancer treatment. Anti-angiogenic effects of methanolic and aqueous extracts of *S. crispus* were examined using *ex vivo* rat aortic ring assay and compared to a positive control, suramin [13]. The anti-angiogenic activity was observed in both extracts with aqueous extract showing greater inhibitory action (16.67%) than the methanolic extract (6.25%) on angiogenesis.

The mechanism of action of *S. crispus* through regulation of some tumour marker enzymes was investigated by another study using animal model. The cancer suppressive activity of *S. crispus* leaf extract was evaluated in diethyl nitrosamine/2-acetylaminofluorene (DEN/AAF)–induced hepatocarcinogenesis [48] in which *S. crispus* extract (1, 2.5, 5 and 7.5%) were supplemented in the drinking water of cancer-induced and control rats. The inhibitory effect was graded based on the histological evaluation and measurements of tumour marker enzymes, glutathione S-transferase (GST) and uranyl diphosphate glucoronyl transferase (UDPGT). Oral administration of *S. scripus* at 5% was shown to be most effective in reducing the severity of liver cancer by decreasing cellular dysplasia [49] as well as GST and UDPGT activities [50]. It also reduced levels of gamma-glitamyl transpeptidase (GGT), alkaline phosphatase (ALP) and glutathione (GSH) significantly [17].

In addition, the chemopreventive mechanism exerted by *S. crispus* on hepatocellular carcinoma was found to be related to the inhibition of microsomal aniline hydroxylase (AH) activity which is involved in the activation of carcinogen DEN [48]. Although the study showed the potential of *S. crispus* in bringing down the tumour marker enzymes, the rats, however, did not fully recover from the liver tumour. The limitations of the study include short experimental duration and choice of animal species/strain.

Strain-related differences in responsiveness to the treatment as well as in susceptibility to the carcinogens DEN and AAF could be a misleading factor during result interpretation. For instance, the effects observed could be attributed to the effectiveness of plant extract tested or was simply because of strain resistance. Moreover, genetic variability among strains (in the study Sprague-Dawley rat, which is an outbred strain was used), dietary and environmental factors could contribute to the polymorphic differences in drug absorption, metabolism and excretion. Thus, the above-mentioned concerns should be considered to improve the study. Other considerations include interspecies differences, correlation to body weight and pharmacokinetic variables are required in designing animal studies to ensure good prediction on results.

The above studies have consistently showed that the extracts of *S. crispus* have potential anticancer activities both *in vitro* and *in vivo*; and it could be further explored for the development of chemotherapeutic drug.

Combinational therapy has become a trend in anti-cancer treatment in which a few different dugs were used together in lower dose to achieve high efficiency and low toxicity. Tamoxifen has been reported to stimulate apoptosis through modulation of signalling proteins via estrogen receptor (ER)-independent pathway [51, 52] but at the expense of high dosage-related toxicity. Therefore, tamoxifen is not a first-choice drug prescribed for ER-negative breast cancers which are generally more aggressive and have poor prognosis [53]. In this regard, combining tamoxifen with other chemotherapeutic drugs was performed to enhance its efficacy. A study was conducted recently and it revealed a thrilling outcome that co-treatments of *S. crispus* subfraction (SCS) and tamoxifen exhibited synergistically cytotoxic effect in both the ER-responsive MCF-7 and non-responsive MDA-MB-231 breast cancer cells [32]. When incubated with SCS, 2.5 and 5.0 μM of tamoxifen induced 90% of MCF-7 cell death and 85 to 90% of MDA-MB-231 cell death at 24 hours; and this is similar to the effects achievable through high concentration of tamoxifen (15 μM) alone. This suggests the possibility of using sub-optimal doses of tamoxifen to attain the desired cytotoxic effects with reduced side effects and delayed drug resistance. The combinational treatment activated initiator caspases 8 and 9, suggesting the treatment targeted both intrinsic and extrinsic pathways. The increased caspases 8 and 9 levels were evidenced by stronger fluorescent signals detected in cells treated

with combination treatment. In short, the study showed the future potential of *S. crispus* as an anti-cancer agent, particularly its role in combinational therapy. Nevertheless, further studies are required to determine the effectiveness and safety of the combination treatment before clinical trials.

Antioxidant Properties

Oxidative stress, induced by oxygen radicals, is a key feature in various degenerative diseases such as cancer, gastric ulcer and atherosclerosis. Therefore, antioxidant has a significant protective role in preventing the initiation, promotion and progression of carcinogenesis by free radical scavenging and catalytic metal chelation, thus exerting a protective effect on DNA and gene expression.

The antioxidant activity of various types of tea prepared from *S. crispus* leaves was evaluated *in vitro* using Ferric Reducing/ Antioxidant Power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assays and compared to green and black teas [54]. Green tea was endowed with the best antioxidant activity, followed by black tea, *S. crispus* unfermented tea (old), *S. crispus* unfermented tea (young), *S. crispus* fermented tea (old) and *S. crispus* fermented tea (young).

The antioxidant activity of *S. crispus* tea is probably attributed to the polyphenol and flavonoid in tea such as catechins. Polyphenols can chelate transition metal ions, scavenge molecular species of active oxygen, and can inhibit lipid peroxidation by trapping the lipid alkoxyl radical while catechins has many hydroxy groups to donate hydrogen thus making it an efficient radical scavenger [55]. Positive correlation between total phenolic content (TPC) and the DPPH inhibition was established in previous studies [56, 57], suggesting the role of phenolic compounds as antioxidant agents. Overall, it seems that unfermented tea from older leaves naturally contain higher antioxidant activity due to the accumulation of phenolic content over time as well as better preservation of phenolic compounds which is unaltered by fermentation process [58]. These have suggested the importance of tea preparation methods for better preservation of the antioxidant capacity of *S. crispus*.

Variation in the yield of TPC in *S. crispus* extracts clearly depends upon the methods and choices of solvent for extractions. Previous study showed that highly polar medium such as methanol extract gave the best yield of TPC, followed sequentially by ethyl acetate, dichloromethane and hexane [59]. Muslim et al. (2010) showed that at optimal concentration of 100 μg/ml, methanolic and aqueous extracts displayed 90.28% and 89.06% xanthine oxidase inhibition respectively [13]. Results also showed that both aqueous and methanolic extracts were able to quench DPPH and the scavenging activity of aqueous extract was more than the methanolic extract. However, when the extracts were tested for their antioxidant activity with β-carotene-linoleate model system, weak responses were observed. This indicates that the antioxidant action of *S. crispus* is dependent on its hydrogen donating ability (as evidenced by DPPH assay) but not the ability of preventing the bleaching of β-carotene by linoleic acid.

Essential oil of *S. crispus* was also being evaluated for its antioxidant property. It showed to possess higher antioxidant activity compared to α-tocopherol, a potent antioxidant, but its activity was lower than the other plant *Lawsonia inerme*, which was also being tested in the same study [60].

The antioxidant property of *S. crispus* methanolic extracts may be due the presence of phytosterols, such as α-sitosterol, campesterol, phytol and stigmasterol. Phytosterols are structurally similar to cholesterol but are characterized by an extra ethyl (sitosterol) or methyl group (campesterol) in the side chain [61, 62]. Most phytosterols contain 28 or 29 carbons and one or two carbon-carbon double bonds, typically one in the sterol nucleus and sometimes a second in the alkyl side chain. The effectiveness of antioxidant increases with the number of double bonds. β-Sitosterol has been found to exhibit greatest antioxidant activity through the scavenging of free radicals such as DPPH and superoxide radical [63] and the elevation of antioxidant enzyme activities (superoxide dismutase, SOD and glutathione peroxidase, Gpx) in oxidative stress-induced macrophages [64]. Campesterol and ß-sitosterol are better antioxidants compared to stigmasterol in terms of prevention of methyl linoleate oxidation in solution [65] as well as scavenging of superoxide anion and hydrogen peroxide [66]. Phytol is a branched-chain unsaturated alcohol and its antioxidant properties may be related to the hydroxyl group (OH) present in its molecule which is capable of reacting with a free radical and donate hydrogen atoms with an unpaired electron (H·). Taken together, the above studies provide evidences that phytosterol and its components chemically act as a potential antioxidant and a modest radical scavenger.

Agents with antioxidant activity may have cancer preventive effects by reducing the reactive oxygen species (ROS) in cells as ROS is known to cause carcinogenesis. Excessive levels of ROS have been associated with a number of malignancies such as chronic lymphocytic leukemia and Burkitt's lymphoma [67]. As such, *S. crispus* which is a potent antioxidant may be considered as a chemopreventive agent. Although inhibition of ROS formation is desirable for the prevention of cancer development, utilizing pro-oxidant agents to induce oxidative stress has emerged as an attractive alternative anticancer strategy [68]. The pro-oxidant agents killed cancer cells when they were given at a dose that causing high level of ROS. For example, curcumin promoted the death of cutaneous T-cell lymphoma through the induction of oxidative stress [69]. It is important to note that concentration used is crucial to determine the role of a compound as a cancer chemopreventive or chemotherapeutic agent [70]. In short, *S. crispus* may be a chemopreventive agent when given at lower doses by acting as an antioxidant and acts as chemotherapeutic agent if

given at higher doses that sufficiently generating cytotoxic level of ROS.

Considering the strong antioxidant effect of *S. crispus*, further works are required to identify the exact active compound in the extract. This would help in discovering potent anti-oxidative agent from the plant. In addition, *in vivo* sub-acute and chronic toxicity studies are required to be carried out in the future to determine the long term effects of administration of *S. crispus* to validate the potential use of *S. crispus* as a source of anti-oxidant agent.

Anti-Diabetic Effects

Diabetes mellitus is a prevalent metabolic disorder characterized by chronic hyperglycemia resulting from defects in insulin secretion and/or action; and is frequently associated with complications such as retinopathy, end-stage renal failure, neuropathy and 2-4 fold increase in the risk of cardiovascular heart disease [71]. Common oral hypoglycemic agents used such as sulfonylurea are associated with intentional and accidental hypoglycemic poisoning related to overdosing. This necessitates a search for safer and more effective hypoglycemic agent that could help to control blood glucose level in long term with lower side effects. One of the examples is by utilizing *S. crispus* plant extracts.

The anti-hyperglycemic effects of fermented and unfermented *S. crispus* aqueous extracts have been assessed in normal and streptozotocin-induced diabetic rats for 21 days [72]. It revealed that supplementation of the fermented and unfermented extracts of *S. crispus* was correlated with significant reduction of blood glucose in hyperglycemic rats. Furthermore, improvement of lipid profile with decreased levels of total cholesterol, triglycerides, low-density lipoprotein (LDL) and increased high-density lipoprotein (HDL) was observed in *S. crispus* extract-treated normal and diabetic groups. In the study, *S. crispus* shown to possess more superior and prominent anti-hyperglycemic and hypolipidemic effect in comparison to the antidiabetic drug glibenclamide.

The hypoglycemic effect of *S. crispus* is exerted through epicatechin, a flavonoid collectively grouped under catechins, which was present in high amount in the leaves [10]. Epicatechin has been reported to have insulin-like activity [73], functional restoration [74] and tissue protective effect [75] on pancreatic beta-islet cells of alloxan-induced diabetic rats. Type II diabetes is frequently associated with hypercholesterolemia, lower HDL and higher LDL levels. Plant sterols (stigmasterol and ß-sitosterol) that are extracted from the leaves of *S. crispus* [76] may serve as cholesterol-lowering agents by competing with cholesterol and thereby decreasing cholesterol absorption in intestine [77].

The anti-hyperglycemic mechanism of *S. crispus* is proposed to be mediated by the regulation of endogenous antioxidant enzymes GPx and SOD [78]. From the study conducted by Norfarizan-Hanoon et al. (2009), SOD and GPx levels increased consistently and significantly in diabetic and normal rats treated with *S. crispus* juice at different doses of 1.0, 1.5 and 2.0 mL kg-1 b.wt for 30 days. There is correlation between the depletion of antioxidant capacity and glycemic control in type II diabetes [79]. Persistent hyperglycaemia in diabetes may cause oxidative stress by disrupting the balance between antioxidant protective mechanism and generation of ROS. The increased formation of ROS through glucose autoxidation and non-enzymatic glycation of protein will lead to depletion of antioxidant enzymes [80]. The subsequent impaired antioxidant defence and enhanced lipid peroxidation process are attributed for most of the diabetic complications [81]. Therefore, by increasing the amount of endogenous antioxidant enzymes which prevent oxidation by reducing the rate of chain initiation, *S. crispus* may exert a protective effect on tissues against the destructive reactions of superoxide radicals. Moreover, the high antioxidant activity of *S. crispus* tea leaves protected pancreas from oxidative stress by free radicals scavenging. The oxidative stress may mediate pathogenesis of atherosclerosis through endothelial disruption [82], which in turn promotes platelet adhesion and aggregation [83] as well as vascular lipid peroxidation and increases expression of adhesion molecule on intimal surface facilitating leucocyte infiltration. The subsequent plaque formation leads to partial occlusion of blood vessel and hypertension. Hence, the antioxidant property of *S. crispus* contributed to reducing risk and mortality of cardiovascular diseases associated with diabetes. However, further studies including clinical trials are required to prove the therapeutic efficacy of *S. crispus* in diabetic patients.

Wound Healing Effects

S. crispus showed to have beneficial effect in promoting wound healing in previous studies. Topical application of *S. crispus* ethanolic extract accelerated the rate of wound healing by inducing angiogenesis and increased collagen formation [84]. Intrasite gel as standard control and gum acacia as placebo control were used in the study. *S. crispus* resulted in significantly smaller wound areas after 5 and 10 days treatment. After 10 days, the wounds dressed with *S. crispus* at concentration 100 and 200 mg/ml showed to have 81.25 and 92.00% healing respectively. Meanwhile, the healing by placebo control was only 70%. Notable difference was also seen after 5 days treatment, in which the *S. crispus*-treated wounds were more than 50% healed while the placebo control group was just 35% better. Besides that, the healing time was considerably shorter for intrasite gel-treated and *S. crispus*-treated wounds, when compared to placebo. Finally, healed wound treated with *S. crispus* leaf extract revealed less scar width at wound closure and showed better histopathological features in terms of increased re-epithelialisation, decreased inflammatory infiltration and more collagen deposition with angiogenesis than wounds treated with gum acacia.

Another investigation was carried out to evaluate the effect of *S. crispus* juice at different doses (70, 105 and 140 mg/kg b.wt) on diabetes wound [85]. It was reported that *S. crispus* juice aided

in wound healing, in which the percentage of healing in both hyperglycemic and normal rats significantly increased at day 3 and 7 after treatment. The findings may impose some medical implications for diabetic patients who often develop serious complications such as foot ulceration with subsequent amputation in 5-15% of the cases due to delayed wound healing.

The wound healing property of *S. crispus* is believed to be correlated with the presence of anti-microbial compound. An ester glycoside compound of caffeic acid extracted from *S. cripus* is known to have antimicrobial effects when used externally [86], which appears to be responsible for the improved healing activity. Infected wound healed slower compared to clean wound. Bacterial infections exacerbate inflammatory response and delay wound healing by impeding angiogenesis, interference with collagen formation and production of toxins, metabolic products and inflammatory mediators. Infected wound may gradually become chronic with prolonged inflammatory phase. The resulting imbalance of matrix metalloproteases (MMPs) and tissue inhibitor of metalloproteinases (TIMPs) can cause growth factors in chronic wound to be rapidly degraded [87, 88]. Therefore, inhibition of infections may shorten the inflammatory process needed to clear away microbial agents and provide an optimal microenvironment for wound healing.

The antioxidant effect of *S. crispus* may also play a significant role in protecting tissue from oxidative damage and hence promoting wound healing. Oxidative stress results from the excessive production of ROS such as hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\bullet -}$), and hydroxyl radical ($^{\bullet}OH$) has been thought to be involved in the pathogenesis of chronic wounds [89]. These highly unstable molecules damage cells and alter cell membrane and protein structure by lipid peroxidation and oxidative modification. Previous studies reported a significant elevation of the allantoin: uric acid percentage ratio, a marker of oxidative stress, in fluid exudate from chronic leg ulcers [90] as well as increased oxidative lipid damage and nitrative protein damage, as measured by biomarkers F2-isoprostanes and 3-nitrotyrosine respectively in mouse wounds [91].

The use of topical formulations of *S. crispus* for treatment of wounds decreases the risk of adverse effects and potential drug-drug interactions associated with systemic medications. However, the current evidences of the potential effect of *S. crispus* on wound healing are based entirely on animal studies which are limited by small sample sizes and short experimental duration. Future research development should improve on these aspects. Future works could also involve the investigation on various types of wounds such as infected, anastomotic and ischemic wounds in animal studies. Comparison of the healing effect of *S. crispus* with some commercialized drugs may also be carried out as the information available is very limited.

Anti-Thrombosis Effects

The inhibitory effects of *S. crispus* on platelet aggregation and coagulation have been discovered previously [92]. The coagulation pathway can be divided into the extrinsic and the intrinsic pathways. In brief, the extrinsic coagulation is initiated *in vivo* by tissue factor exposure following vascular injury which leads to activation of factor VIIa (FVIIa) whereas intrinsic pathway is activated when factor XII (Hageman factor) contacts with collagen underlying the endothelium of blood vessel wall. The activated factor then catalyses the activation of a larger amount of the next factor down the coagulation cascade, leading eventually to the activation of a common factor X (FXa) in both pathways. FXa hydrolyzes and activates prothrombin to thrombin. Subsequent cleavage of soluble fibrinogen to insoluble fibrin by thrombin forms a mesh that along with the aggregation of platelets can result in formation of a more stable fibrin clot.

Seventy percent methanolic extract and water fraction of *S. crispus* inhibited coagulation by affecting the intrinsic pathway with prolonged activated partial thromboplastin time (aPTT) and unchanged prothrombin time (PT). Additionally, it was observed that water fraction was far more active since its effective concentration in prolonging aPTT is lower than the 70% methanolic extract, suggesting the bioactive compounds responsible for the anticoagulant activities were more polar in nature. This indicates that *S. crispus* may function as a potential antithrombotic agent for thrombolytic therapy by inhibiting platelet aggregation and delaying coagulation time.

Conclusion

To date, the beneficial effects of *S. crispus* on anticancer, anti-hyperglycemic, antioxidant, wound healing and anticoagulant activities have been evaluated in a number of *in vitro* and animal studies. The recent progress on both studies provides significant evidences that *S. crispus* could be explored further for therapeutic potential. However, as most of the scientific reports are based on animal experiments, clinical trials are necessary to prove its efficacy. Long term safety concerning the consumption of *S. crispus* should also be emphasized. Further works should be focused on the isolation, purification and identification of biologically active compounds in *S. crispus* so that new component responsible for the medicinal effects could be identified soon.

References

1. Mishra BB, Tiwari VK (2011) Natural products: an evolving role in future drug discovery. Eur J Med Chem 46(10): 4769-4807.

2. Cragg G, Newman D (2005) Biodiversity: A continuing source of novel drug leads. Pure and applied chemistry 77(1): 7-24.

3. Butler MS (2004) The role of natural product chemistry in drug discovery. J Nat Prod 67(12): 2141-2153.

4. Wall ME, Wani MC (1995) Amptothecin and taxol: discovery to clinic-thirteenth Bruce F. Cain Memorial Award Lecture. Cancer Res 55(4): 753–760.

5. Noble RL, Beer CT, Cutts JH (1958) Role of chance observations in chemotherapy: Vinca rosea. Ann N Y Acad Sci 76(3): 882-894.

6. Sunarto PA (1997) Materia medica Indonesia. (1stedn), Penerbitan Directional Jenderal Pengawasan Obat dan Makanan, Jakarta, Indonesia.

7. Perry L, Metzger J (1980) Medicinal plants of East and Southeast Asia. (1stedn), MIT Press, Cambridge.

8. Samuel AJ, Kalusalingam A, Chellappan D, Gopinath R, Radhamani S, et al. (2010) Ethnomedical survey of plants used by the Orang Asli in Kampung Bawong, Perak, West Malaysia. J Ethnobiol Ethnomed 6(5).

9. Noraida A (2005) Penyembuhan Semula Jadi dengan Herba. PTS Millennia Sdn. Bhd, Kuala Lumpur, Malaysia.

10. Ismail M, Manickam E, Danial A, Rahmat A, Yahaya A (2000) Chemical composition and antioxidant activity of Strobilanthes crispus leaf extract. J Nutr Biochem 11(11-12): 536-542.

11. Rahmat A, Edrini S, Md. Akim A, Ismail P, Yap Yun Hin T, et al. (2006) Anticarcinogenic properties of Strobilanthes crispus extracts and its compounds in vitro. International Journal of Cancer Research 2(1): 47-49.

12. Endrini S, Rahmat A, Ismail P, Taufiq-Yap Y (2007) Comparing of the cytotoxicity properties and mechanism of Lawsonia inermis and Strobilanthes crispus extract against several cancer cell lines. Journal of Medical Sciences 7(7): 1098-1102.

13. Muslim NS, Ng KW, Itam A, Nassa ZD, Abdul Majid AMS, et al. (2010) Evaluation of cytotoxic, anti-angiogenic and antioxidant properties of standardized extracts of Strobilanthes crispus leaves. International Journal of Pharmacology 6(5): 591-599.

14. Ng KW, Salhimi SM, Majid AM, Chan KL (2010) Anti-angiogenic and cytotoxicity studies of some medicinal plants. Planta Med 76(09): 935-940.

15. Yaacob NS, Hamzah N, Kamal N, Abidin S, Lai C, et al. (2010) Anticancer activity of a sub-fraction of dichloromethane extract of Strobilanthes crispus on human breast and prostate cancer cells in vitro. BMC complementary and alternative medicine 10(1): 42.

16. Chong HZ, Rahmat A, Yeap SK, Akim A, Alitheen NB, et al. (2012) In vitro cytotoxicity of Strobilanthes crispus ethanol extract on hormone dependent human breast adenocarcinoma MCF-7 cell. BMC Complement Altern Med 12(1): 35.

17. Suherman J, Asmah R, Fauziah O, Patimah I, Nor Haslinda A (2004) Effect of Strobilanthes crispus on tumour marker enzymes and glutathione during chemical hepatocarcinogenesis in the rat. Pakistan Journal of Biological Sciences 7(6): 947-951.

18. Endrini S, Suherman J, Rahmat A (2007) Effects of Strobilanthes crispus extract on the apoptotic pathway of human liver carcinoma cell lines. Jurnal Kedorkteran Yarsi 15(1): 1-5.

19. Hanachi P, Othman F, Rahmat A (2008) Lesion scoring and P450 isoenzyme activity in liver of hepatocarcinogenesis rats treated with Strobilanthes crispus. Iranian J Cancer Prevention 1(1): 12-16.

20. Park C, Moon DO, Rhu CH, Choi BT, Lee WH, et al. (2007) Beta-sitosterol induces anti-proliferation and apoptosis in human leukemic U937 cells through activation of caspase-3 and induction of Bax/Bcl-2 ratio. Biol Pharm Bull 30(7): 1317-1323.

21. Awad AB, Burr AT, Fink CS (2005) Effect of resveratrol and b-sitosterol in combination on reactive oxygen species and prostaglandin release by PC-3 cells. Prostaglandins Leukot Essent Fatty Acids 72(3): 219–226.

22. Awad AB, Chinnam M, Fink CS, Bradford PG (2007) b-Sitosterol activates Fas signaling in human breast cancer cells. Phytomedicine 14(11): 747–754.

23. Moon D, Lee K, Choi YH, Kim G (2007) ß-sitosterol-induced-apoptosis is mediated by the activation of ERK and the down-regulation of Akt in MCA-102 murine fibrosarcoma cells. Int Immunopharmacol 7(8): 1044–1053.

24. McCann SE, Freudenheim JL, Marshall JR, Graham S (2003) Risk of human ovarian cancer is related to dietary intake of selected nutrients, phytochemicals and food groups. J Nutr 133(6): 1937–1942.

25. Rossi D, Gaidano G (2003) Messengers of cell death: apoptotic signaling in health and disease. Haematologica 88(2): 212–218.

26. Cory S, Adams JM (2002) The Bcl2 family: regulators of the cellular life-or-death switch. Nat Rev Cancer 2(9): 647–656.

27. Billen L, Kokoski C, Lovell J, Leber B, Andrews D (2008) Bcl-XL inhibits membrane permeabilization by competing with Bax. PLoS biology 6(6): 147.

28. Wang X (2001) The expanding role of mitochondria in apoptosis. Genes Dev 15(22): 2922-2933.

29. Saelens X, Festjens N, Vande Walle L, van Gurp M, van Loo G, et al. (2004) Toxic proteins released from mitochondria in cell death. Oncogene 23(16): 2861–2874.

30. Gosslau A, Chen KY (2004) Nutraceuticals, apoptosis, and disease prevention. Nutrition 20(1): 95-102.

31. Denault JB, Selvesen GS (2002) Caspases: Keys in the ignition of cell death. Chem Rev 102(12): 4489-4500.

32. Yaacob NS, Kamal NN, Norazmi MN (2014) Synergistic anticancer effects of a bioactive subfraction of Strobilanthes crispus and tamoxifen on MCF-7 and MDA-MB-231 human breast cancer cell lines. BMC Complement Altern Med 14(1): 252.

33. Toshiyuki M, Reed JC (1995) Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. Cell 80(2): 293-299.

34. Guseva NV, Taghiyev AF, Rokhlin OW, Cohen MB (2002) Contribution of death receptor and mitochondrial pathways to Fas-mediated apoptosis in the prostatic. Prostate 51(4): 231–240.

35. Dang CV (1999) c-Myc target genes involved in cell growth, apoptosis, and metabolism. Mol Cell Biol 19(1): 1-11.

36. van Es JH, Barker N, Clevers H (2003) You Wnt some, you lose some: oncogenes in the Wnt signaling pathway. Curr Opin Genet Dev 13: 28–33.

37. Kolligs FT, Kolligs B, Hajra KM, Hu G, Tani M, et al. (2000) γ- Catenin is regulated by the APC tumor suppressor and its oncogenic activity is distinct from that of β-catenin. Genes Dev 14(11): 1319–1331.

38. Boxer LM, Dang CV (2001) Translocations involving c-myc and c-myc function. Oncogene 20(40): 5595–5610.

39. Dalla-Favera R, Bregni M, Erikson J, Patterson D, Gallo RC, et al. (1982) Human c-myc onc gene is located on the region of chromosome 8 that is translocated in Burkitt lymphoma cells. Proc Natl Acad Sci USA 79(24): 7824-7827.

40. Bahram F, von der Lehr N, Cetinkaya C, Larsson LG (2000) c-Myc hot spot mutations in lymphomas result in inefficient ubiquitination and decreased proteasome-mediated turnover. Blood 95(6): 2104–2110

41. Gregory MA, Hann SR (2000) c-Myc proteolysis by the ubiquitin-proteasome pathway: stabilization of c-Myc in Burkitt's lymphoma cells. Mol Cell Biol 20(7): 2423–2435.

42. Nesbit CE, Tersak JM, Prochownik EV (1999) MYC oncogenes and human neoplastic disease. Oncogene 18(19): 3004-3016.

43. D'Agnano I, Valentini A, Fornari C, Bucci B, Starace G, et al. (2001) Myc down-regulation induces apoptosis in M14 melanoma cells by increasing p27Kip1 levels. Oncogene 20(22): 2814-25.

44. Watson PH, Pon RT, Shiu RPC (1991) Inhibition of c-myc expression by phosphorothioate antisense oligonucleotide identifies a critical role for c-myc in the growth of human breast cancer. Cancer Res 51: 3996-4000.

45. Wang Y, Liu S, Zhang G, Zhou C, Zhu H, et al. (2005) Knockdown of c-Myc expression by RNAi inhibits MCF-7 breast tumor cells growth in vitro and in vivo. Breast Cancer Res 7(2): 220--228.

46. Steiner MS, Anthony CT, Lu Y, Holt JT (1998) Antisense c-myc retroviral vector suppresses established human prostate cancer. Hum Gene Ther 9: 747-755.

47. Balaji KC, Koul H, Mitra S, Maramag C, Reddy P, et al. (1997) Antiproliferative effects of c-myc antisense oligonucleotide in prostate cancer cells: a novel therapy in prostate cancer. Urology 50: 1007-1015.

48. Fauziah O, Hanachi P, Yogespiriya S, Asmah R (2005) Evaluation of lesion scoring and aniline hydroxylase activity in hepatocarcinogenesis rats treated with Strobilanthes crispus. Journal of Medical Sciences 5(1): 26-30.

49. Elizabeth M, Fauziah O, Asmah R, Maznah I, Asmah Y, et al. (2000) Changes in the morphology of induced-liver cancer treated with Strobilanthes crispus. Ann Microscopy 1: 23-32.

50. Jaksa S, Rahmat A, Othman F, Ismail P, Mansor S (2005) Effect of Strobilanthes crispus on the histology and tumour marker enzymes in rat liver during hepatocarcinogenesis. Journal of Medical Sciences 5(2): 130-135.

51. Fattman CL, An B, Sussman L, Dou QP (1998) p53-Independent dephosphorylation and cleavage of retinoblastoma protein during tamoxifen-induced apoptosis in human breast cancer carcinoma cells. Cancer Lett. 130: 103–113.

52. Ferlini C, Scambia G, Distefano M, Filippinin P, Isola G, et al. (1997) Synergistic antiproliferative activity of tamoxifen and docetaxel on three oestrogen receptor-negative cancer cell lines is mediated by the induction of apoptosis. Br J Cancer 75: 884–891.

53. Sheikh MS, Garcia M, Pujol P, Fontana JA, Rochefort H (1995) Why are estrogen-receptor-negative breast cancers more aggressive than the estrogen-receptor-positive breast cancers? Invasion and Metastasis 14: 329–336.

54. Bakar M, Teh A, Rahmat A, Hashim N, Othman F, et al. (2006) Antiproliferative properties and antioxidant activity of various types of Strobilanthes crispus tea. International Journal of Cancer Research 2(2): 152-158.

55. Rice-Evans C, Miller N, Paganga G (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free radical biology and medicine 20(7): 933-956.

56. Gorinstein S, Vargas O, Jaramillo N, Salas I, Ayala A, et al. (2007) The total polyphenols and the antioxidant potentials of some selected cereals and pseudocereals. Eur. Food Res Technol. 225: 321-328.

57. Qader S, Abdulla M, Chua L, Najim N, Zain M, et al. (2011) Antioxidant, total phenolic content and cytotoxicity evaluation of selected Malaysian plants. Molecules 16(4): 3433-3443.

58. Abu Bakar MF, Teh AH, Rahmat A, Hashim N, Othman F, et al. (2004) Antioxidant tea from leaves of Strobilanthes crispus. J. Tropical Med. Plants 5: 199-204.

59. Ismail M, Bagalkotkar G, Iqbal S, Adamu H (2012) Anticancer properties and phenolic contents of sequentially prepared extracts from different parts of selected medicinal plants indigenous to Malaysia. Molecules 17(5): 5745-5756.

60. Rahmat A, Edrini S, Ismail P, Yap T, Abu Bakar M (2006) Chemical constituents, antioxidant activity and cytotoxic effects of essential oil from Strobilanthes crispus and Lawsonia inermis. Journal of Biological Sciences 6(6): 1005-1010.

61. Grundy SM (1983) Absorption and metabolism of dietary cholesterol. Ann Rev Nutr 3: 71–96.

62. Normen L, Dutta P, Lia A, Andersson H (2000) Soy sterol esters and ß-sitostanol ester as inhibitors of cholesterol absorption in human small bowel. Am J Clin Nutr 71: 908–913.

63. Karan S, Mishra S, Pal D, Mondal A (2012) Isolation of beta-sitosterol and evaluation of antidiabetic activity of Aristolochia indica in alloxan-induced diabetic mice with a reference to in vitro antioxidant activity. Journal of Medicinal Plants Research 6(7): 1219-1223.

64. Vivancos M, Moreno JJ (2005) ß-Sitosterol modulates antioxidant enzyme response in RAW 264 7 macrophages. Free Radic Biol Med 39: 91-97.

65. Yoshida Y, Niki E (2003) Antioxidant effects of phytosterol and its components. Journal of nutritional science and vitaminology 49(4): 277-280.

66. Lim YM (2002) Anti-Tumour Promoting Activity of Selected Malaysian Vegetables and Fruits, and Identification of Anti-Tumour Promoting and Antioxidant Compounds from Coleus Tuberosus, Benth (Ubi Kemili). Universiti Putra Malaysia, Malaysia.

67. Halliwell B (2007) Oxidative stress and cancer: have we moved forward? Biochem J 401: 1-11.

68. Martín-Cordero C, León-González AJ, Calderón-Montaño JM, Burgos-Morón E, López-Lázaro M (2012) Pro-Oxidant natural products as anticancer agents. Current Drug Targets 13: 1006-1028.

69. Khan MA, Gahlot S, Majumdar S (2012) Oxidative stress induced by curcumin promotes the death of cutaneous T-cell lymphoma (HuT-78) by disrupting the function of several molecular targets. Mol Cancer Ther 11(9): 1873–1883.

70. Lopez-Lazaro M (2008) Anticancer and carcinogenic properties of curcumin: considerations for its clinical development as a cancer chemopreventive and chemotherapeutic agent. Mol Nutr Food Res 52(1): S103-27.

71. Amos AF, McCarty DJ, Zimmet P (1997) The rising global burden of diabetes and its complication: Estimates and projections to the year 2010. Diabetic Med 14(5): S1-S85.

72. Fadzelly ABM, Asmah R, Fauziah O (2006) Effects of *Strobilanthes crispus* tea aqueous extracts on glucose and lipid profile in normal and streptozotocin-induced hyperglycemic rats. Plant Foods Hum Nutr 61(1): 7-11.

73. Ahmad F, Khalid P, Khan MM, Rastogi AK, Kidwai JR (1989) Insulin like activity in (-)epicatechin. Acta Diabetol Lat 26: 291-300.

74. Chakravarthy BK, Gupta S, Gode KD (1982) Functional beta cell regeneration in the islets of pancreatic in alloxan induced diabetic rats by (-)epicatechin. Life Sci 31(24): 2693-2697.

75. Chakravarthy BK, Gupta S, Ghambir SS, Gode KD (1981) Pancreatic beta cell regeneration in rats by (-) epicatechin. Lancet 2(8249): 759-760.

76. Abdah MA, Asmah R, Taufiq-Yah YH , Patimah I (2004) Flow cytometry analysis on the effects of stigmasterol on the breast cancer cell lines, MCF-7. Malays J Clin Biochem 4(1): 64-68.

77. Ellegard L, Bosaeus I, Anderson H (2000) Will recommended changes in fat and fibre intake affect cholesterol absorption and sterol excretion? An ileostomy study. Eur J Clin Nutr 54: 306-313.

78. Norfarizan NA, Asmah R, Rokiah MY, Fauziah O, Faridah H (2009) Antihyperglycemic, hypolipidemic and antioxidant enzymes effect of *Strobilanthes crispus* juice in normal and streptozotocin-induced diabetic male and female rats. International Journal of Pharmacology 5(3): 200-207.

79. Opara EC, Abdel-Rahman E, Soliman S, Kamel WA, Souka S, et al. (1999) Depletion of total antioxidant capacity in type II diabetes. Metabolism 48: 1414-1417.

80. Maritim AC, Sanders RA, Watkins JB (2003) Diabetes, oxidative stress and antioxidants. A Review. J Biochem Mol Toxicol 17: 24-38.

81. Palanduz S, Ademoglu E, Gokkusu C (2001) Plasma antioxidants and type 2 diabetes mellitus. Pharmacology 109: 309-318.

82. Munzel T, Sinning C, Post F, Warnholtz A, Schulz E (2008) Pathophysiology, diagnosis and prognostic implications of endothelial dysfunction. Ann Med 40: 180–196.

83. Zorio E, Gilabert-Estellés J, España F, Ramón LA, Cosín R, et al. (2008) Fibrinolysis: the key to new pathogenetic mechanisms. Curr Med Chem 15: 923-929.

84. Al-Henhena N, Mahmood A, Al-Magrami A, Nor Syuhada A, Zahra A, et al. (2011) Histological study of wound healing potential by ethanol leaf extract of *Strobilanthes crispus* in rats. J Med Plants Res 5(16): 3666--3669.

85. Norfarizan-Hanoon NA, Asmah R, Rokiah MY, Fauziah O, Faridah H (2009) Effects of *Strobilanthes crispus* juice on wound healing and antioxidant enzymes in normal and streptozotocin-induced diabetic rats. Journal of Biological Sciences 9(7): 662-668.

86. Soediro L, Pellecuer J, Andary C, Privat G (1983) *S. crispus* (L) Bl, I: Pemeriksaan senyawa turunan asam kafeat verbaskosid. Acta Pharma.Indones VIII(I): 1-10.

87. Edwards R, Harding K (2004) Bacteria and wound healing. Current opinion in infectious diseases 17(2): 91-96.

88. Menke N, Ward K, Witten T, Bonchev D, Diegelmann R (2007) Impaired wound healing. Clinics in dermatology 25(1): 19-25.

89. Schafer M, Werner S (2008) Oxidative stress in normal and impaired wound repair. Pharmacological Research 58(2): 165-171.

90. James T, Hughes M, Cherry G, Taylor R (2003) Evidence of oxidative stress in chronic venous ulcers. Wound repair and regeneration 11(3): 172-176.

91. Loo A, Wong Y, Ho R, Wasser M, Du T, et al. (2012) Effects of hydrogen peroxide on wound healing in mice in relation to oxidative damage. PloS one 7(11): 49215.

92. Cheng CL (2009) Pharmacological Evaluation of *Strobilanthes crispus* (L.) Blume. National University of Singapore, Singapore.