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Review Article

A Review of the Secondary Metabolites and Biological Activities of *Tinospora crispa* (Menispermaceae)

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Abstract

Tinospora crispa Beumee, a herbaceous climber, has been widely used in traditional medicine for treating various ailments such as contusion, septicaemia, fever, fracture, scabies and other tropical ulcers. A wide range of secondary metabolites such as alkaloids, diterpenes, flavones, phenolics, and triterpenes have been isolated, some of which have also shown corresponding biological activities. The current review is an update on the reported pharmacological activities and phytochemical constituents of T. crispa.

Keywords: *Antioxidant, Alkaloid, Diterpene, Flavone, Triterpene*

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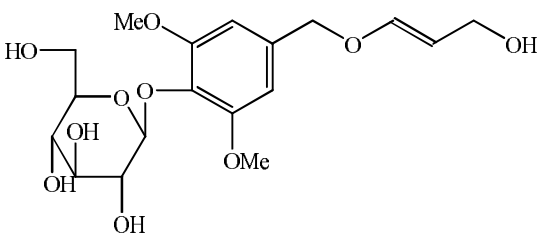
INTRODUCTION

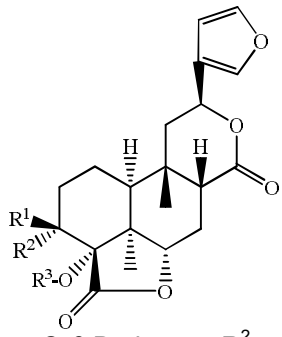
Tinospora crispa Beumee (Menispermaceae) [syn: *T. tuberculata*] or known as “akar patawali” or “akar seruntum” in Malaysia and “Borapet” in Thai and “Da ye ruan jin teng” in Chinese, is a herbaceous climbing plant that is widely distributed in South East Asia, particularly in Vietnam, Thailand, Malaysia, Indonesia and India [1-5]. This medicinal herb has been used in the Thai traditional medicine due to its anti-pyretic, antidiabetic, anti-inflammatory, anti-malarial and health maintaining properties [6]. It is also has been used in Chinese traditional medicine for the treatment of contusion, septicaemia, fever, fracture, scabies and other tropical ulcer related disorders [5,7]. Oral administration of the decoction of the stem of the plant is claimed to possess anti-malarial and anti anthelmintic properties whereas decoction of the whole plant has been used as a postpartum remedy and also a traditional remedy for diabetes among the Malay community [3,8-12]. A poultice of fresh leaves is reported for treatment of wounds and itches [1,13].

PREVIOUS ISOLATION AND CHEMICAL INVESTIGATION

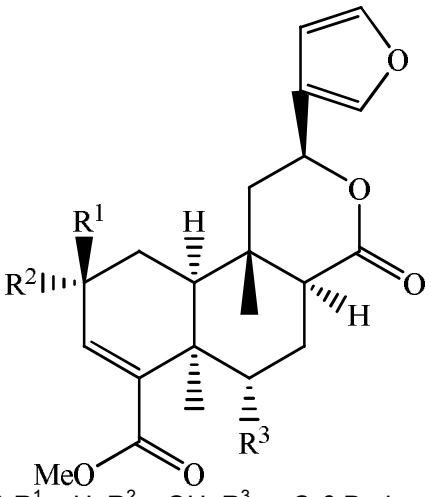
Phytochemical investigation of the stems of *T. crispa* has led to the isolation of *N-trans*-feruloyl tyramine and *N-cis*-feruloyl tyramine from the ethyl acetate fraction as well as a phenolic glucoside, namely, tinotuberide (**1**) from the butanol fraction [14]. In 1985, a diterpene glucoside, borapetoside A (**2**), and its aglycone, borapetol A (**3**) were isolated from the stems of *T. crispa*, followed by the isolation of borapetoside B (**4**), and its aglycone, borapetol B (**5**) on the subsequent year [15-16]. The presence of borapetoside C–H (**6**-**11**) and tinotufolin A–F (**12**-**17**) were identified from the stems of the plant [17-20].



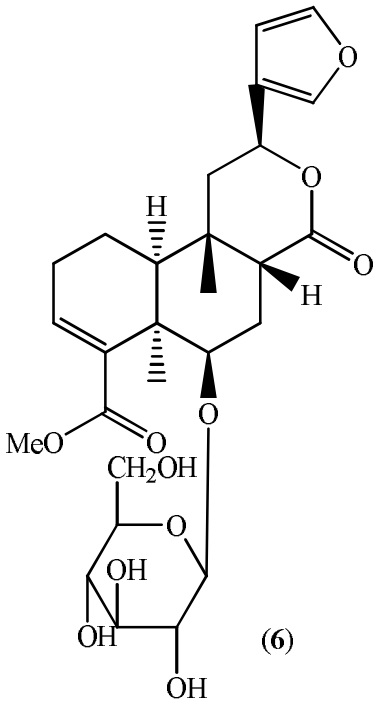
(**1**)



(**2**) R^1 = -*O*-*β*-D-glc.pyr., R^2 = R^3 = H
(**3**) R^1 = OH, R^2 = R^3 = H

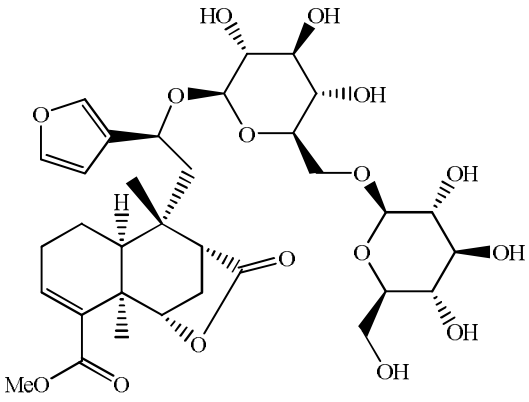


(**4**) R^1 = H, R^2 = OH, R^3 = -*O*-*β*-D-glc.pyr.
(**5**) R^1 = H, R^2 = R^3 = OH



(**6**)

Figure 1: Compound **1** - **6**



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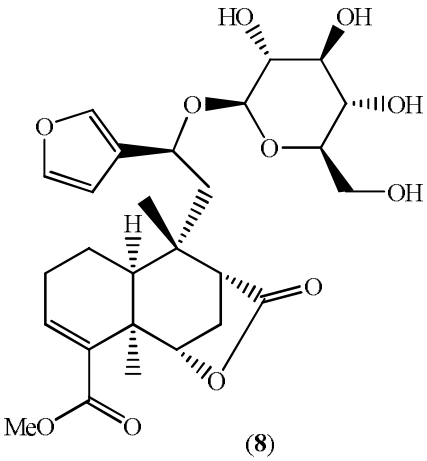

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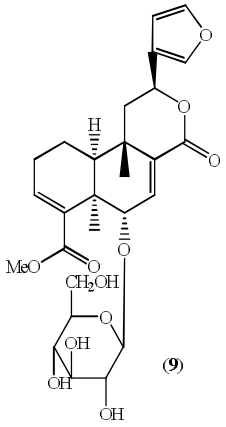


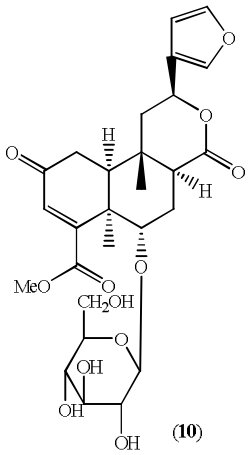
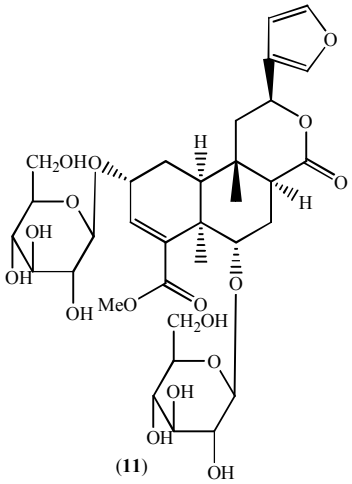
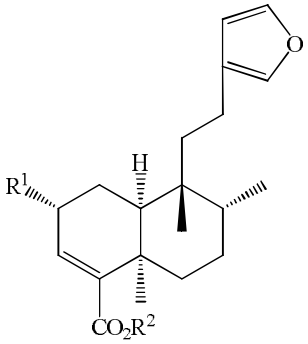
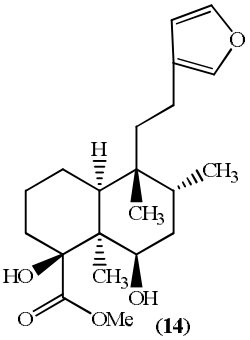
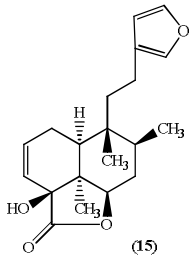


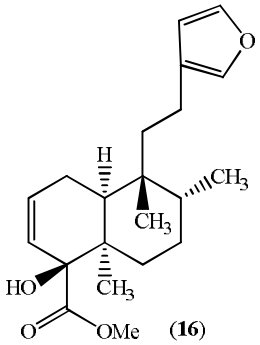
Figure 2: Compounds **7** - **10**











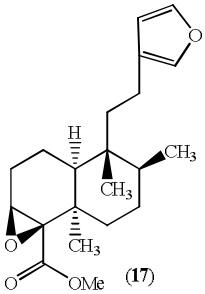


Figure 3: Compounds **11** - **17**

Umi Kalsom & Noor [21] reported from the stems of *T. crispa* five flavone glycosides which were identified as luteolin 4'-methyl ether 7-glucoside, genkwanin 7-glucoside, luteolin 4'-methyl ether 3'-glucoside, diosmetin and genkwanin.

Lin [22] isolated twelve constituents from the methanol extract of the stems of *T. crispa*, which included *β*-sitosterol, stigmasterol, apigenin, N-formylanonaine, N-formylnornuciferine, cycloeucalenol, N-*cis*-feruloyltyramine, N-*trans*-feruloyltyramine, whereas this was the first report of makisteron C, N-acetylanonaine, N-acetylnornuciferine, N-*trans*-caffeoyltyramine from *T. crispa*.

The phytochemical investigations of the stems of *T. crispa* afforded one new aporphine alkaloid, N-formylasimilobine 2-*O*-*β*-D-glucopyranoside (**18**) (Figure 4), along with N-formylasimilobine 2-*O*-*β*-D-glucopyranosyl-(1 → 2)-*β*-D-glucopyranoside (tinoscorside A), N-formylanonaine, N-formyldehydroanonaine, N-formylnomuciferine, N-demethyl-N-formyldehydronornuciferine, magnoflorine, paprazine, N-*trans*-feruloyltyramine and cytidine [23].

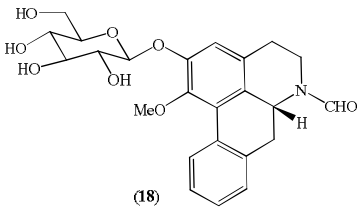
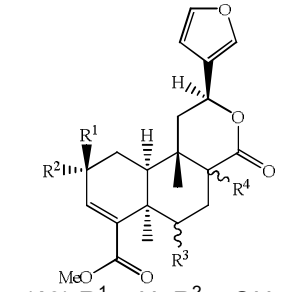


Figure 4: Compound **18**

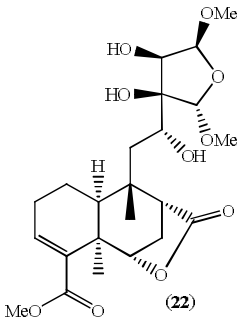
Choudhary *et al.* [24] also isolated nine new *cis*-clerodane-type furanoditerpenoids, namely, (2*R*,5*R*,6*R*,8*S*,9*S*,10*S*,12*S*)-15,16-epoxy-2-hydroxy-6-*O*-{*β*-D-glucopyranosyl-(1→6)-*α*-D-xylopyranosyl}-cleroda-3,13(16),14-trien-17,12-olid-18-oic acid methyl ester (**19**) (Figure 5 and 6), (2*R*,5*R*,6*R*,8*R*,9*S*,10*S*,12*S*)-15,16-epoxy-2-hydroxy-6-*O*-(*β*-D-glucopyranosyl)-cleroda-3,13(16),14-trien-17,12-olid-18-oic acid methyl ester (**20**), (5*R*,6*R*,8*S*,9*R*,10*R*,12*S*)-15,16-epoxy-2-oxo-6-*O*-(*β*-D-glucopyranosyl)-cleroda-3,13(16),14-trien-17,12-olid-18-oic acid methyl ester (**21**), methyl (2*R*,7*S*,8*S*)-8-[(2*S*)-2-(3,4-dihydroxy-2,5-dimethoxytetrahydro-3-furanyl)-2-hydroxyethyl]-2,8-dimethyl-10-oxo-11-oxatricyclo[7.2.1.0]dodec-3-ene-3-carboxylate (**22**), (5*R*,6*R*,8*S*,9*R*,10*S*,12*S*)-15,16-epoxy-2-oxo-6-*O*-(*β*-D-glucopyranosyl)-cleroda-3,13(16),14-trien-17,12-olid-18-oic acid methyl ester (**23**), (2*R*,5*R*,6S,9*S*,10*S*,12*S*)-15,16-epoxy-2-hydroxy-6-*O*-(*β*-D-glucopyranosyl)-cleroda-3,7,13(16),14-tetraen-17,12-olid-18-oic acid methyl ester (**24**), (5*R*,6S,9*S*,10*S*,12*S*)-15,16-epoxy-2-oxo-6-*O*-(*β*-D-glucopyranosyl)-cleroda-3,7,13(16),14-tetraen-17,12-olid-18-oic acid methyl ester (**25**), (3*R*,4*R*,5*R*,6*S*,8*R*,9*S*,10*S*,12*S*)-15,16-epoxy-3,4-epoxy-6-*O*-(*β*-D-glucopyranosyl)-cleroda-3,13(16),14-trien-17,12-olid-18-oic acid methyl ester (**26**) and (1*R*,4*S*,5*R*,8*S*,9*R*,10*S*,12*S*)-15,16-epoxy-4-*O*-(*β*-D-glucopyranosyl)-cleroda-2,13(16),14-triene-17(12),18(1)-diolide (**27**).

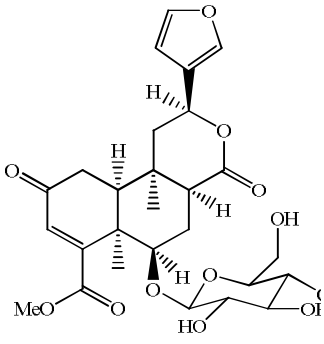


(**19**) R^1 = H, R^2 = OH, R^3= *β*-{*β*-D-glucopyranosyl-(1-6)-*α*-D-xylopyranose}, R^4 = *α*-H

(**20**) R^1 = H, R^2 = OH, R^3= *β*-(*β*-D-glucopyranosyl), R^4 = *β*-H

(**21**) R^1 = R^2 = C=O, R^3= *β*-(*β*-D-glucopyranosyl), R^4 = *α*-H





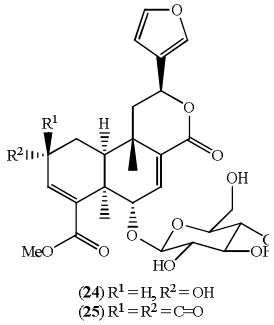
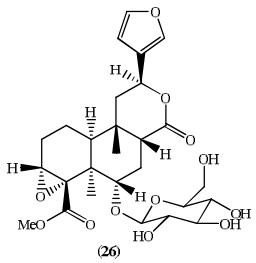


Figure 5: Compounds **19** - **25**



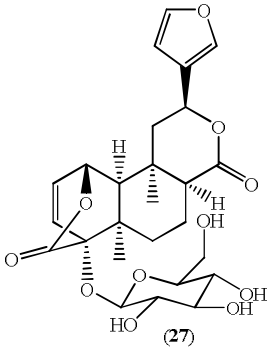


Figure 6: Compounds **26** and **27**

Chung [25] revealed the presence of forty phytochemical constituents, namely, borape-

toside A, borapetoside B, borapetoside C, borapetoside D, borapetoside E, borapetoside F, (2R,5R,6R,8R,9S,10S,12S)-15,16-epoxy-2-hydroxy-6-*O*-(*β*-D-glucopyranosyl)-cleroda-3,13(16),14-trien-17,12-olid-18-oic acid methyl ester, (2R,5R,6S,9S,10S,12S)-15,16-epoxy-2-hydroxy-6-*O*-(*β*-D-glucopyranosyl)-cleroda-3,7,13(16),14-tetraen-17,12-olid-18-oic acid methyl ester, (1R,4S,5R,8S,9R,10S,12S)-15,16-epoxy-4-*O*-(*β*-D-glucopyranosyl)-cleroda-2,13(16),14-triene-17(12),18(1)-diolide, tinocrisposide A, tinocrisposide B, tinocrisposide C, tinocrisposide D, rumphioside A, rumphioside B, rumphioside C, rumphioside F, rumphioside I, borapetol A, borapetol B, borapetic acid A, borapetic acid B, borapetic acid C, N-formylanonaine, N-formylnornuciferine, N-acetylanonaine, N-acetylnornuciferine, N-demethyl-N-formyldehydronuciferine, N-2,3,9-methyltetrahydrocolumbamine, N-*trans*-feruloyl-tyramine, N-*cis*-feruloyltyramine, N-*trans*-caffeoyltyramine, paprazine, *trans*-isoferulic acid, acantrifoside E, 3-hydroy-3-methylglutaryl-acantrifoside E, apigenin, secoisolariciresinol, syringaresinol and cycloeucalenol from the methanol extract of *T. crispa*.

PHYTOCHEMICAL INVESTIGATIONS BASED ON BIO - ACTIVITY

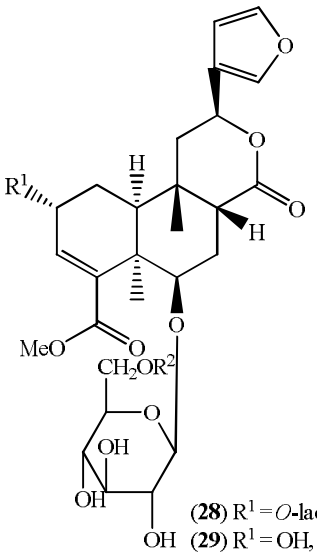
Cavin *et al* [26] examined the dichloromethane extract of *T. crispa* and indicated the presence of vanillin, syringin, *N*-formylannonain, *N*-formylnornuciferin, borapetosides B, C and F, *N-cis*-feruloyltyramine, *N-trans*-feruloyltyramine and secoisolariciresinol. Antioxidant and free-radical scavenging activity of *N-cis*-feruloyltyramine, *N-trans*-feruloyltyramine and secoisolariciresinol were found to exhibit higher antioxidative potency than the synthetic antioxidant butylhydroxytoluene (BHT).

The presence of two triterpenes, namely, cycloeucalenol and cycloeucalenone were reported for the first time from the chloroform extracts of the dried stems of *T. crispa*. Both of the isolated triterpenes further indicated mild cardiotonic effects, where cycloeucalenol showed slight increased in the right atrial contraction and initial reduction followed by 10 % of sustained reduction on the left atria of the rat *in vitro* meanwhile cycloeucalenone, showed slight change on the right and left atrial contraction [27].

Imphanban *et al* [28] isolated an aporphine alkaloid, namely, (-)-N-formylnornuciferine from the stems of *T. crispa*, which revealed *in vitro* cardiotonic activity. Synthesis of the mixture, (±)-N-formylnornuciferine, *via* palladium-catalyzed coupling reaction, indicated significant reduction in the force of contraction and the heart rate.

Bioassay guided fractionation of the n-butanol extract of the stems of *T. crispa* has resulted the isolation of five pharmacologically active compounds which were identified as salsolinol, adenosine, uridine, tyramine and higenamine, along with seven non-active compounds, namely, syringing, borapetoside A, B, D and E, adenine and litcubinine. The investigation of the pharmacologically active compounds on mechanisms of action on the blood pressure and heart rate in anesthetized, normal and reserpinized rats *in vivo* were carried out. The results of investigation further suggested that the cardiovascular responses of the anesthetized rats towards salsolinol, tyramine and higenamine were acted through the stimulation of the adrenoreceptors, whereby uridine and adenosine acted via the purinergic adenosine A_2 and P_2 receptors to decrease blood pressure with a transient decrease of heart rate followed by an increase [29].

Lam *et al* [30] isolated three new diterpenoids, namely, 2-*O*-lactoylborapetoside B (**28**) (Figure 7), 6'-*O*-lactoylborapetoside B (**29**) and tinocrispol A (**30**), along with nine known diterpenoids, identified as borapetosides A-F, borapetols A and B and columbin from the ethanolic extract of *T. crispa* vines. Borapetosides A and C were found to lower the plasma glucose levels in normal and streptozotocin-induced type 1 diabetic mice upon examination on the *in vivo* hypoglycemic activities.



(**28**) R^1 = *O*-lactoyl, R^2 = H
(**29**) R^1 = OH, R^2 = lactoyl

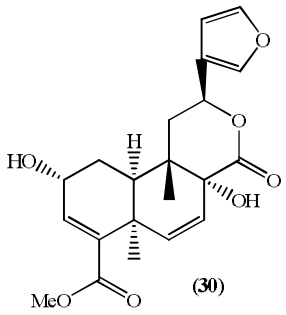


Figure 7: Compounds **28** – **30**

BIOLOGICAL ACTIVITIES AND FORMULATIONS

Antibacterial activity

The antibacterial studies conducted by Al-alusi *et al.* [31] has shown a promising antibacterial activity of *T. crispa* extracts against the Methicillin-resistant *Staphylococcus aureus* (MRSA) as compared to the control, vancomycin.

Antifilarial activity

The aqueous extract of dried stems of *T. crispa*, assessed for *in vitro* antifilarial effects, has indicated moderate activity against the adult worms of subperiodic *Brugia malayi* after the aqueous extract of *Xylocarpus granatum* and dried leaves of *Andrographis paniculata*, whereby the value of relative movability values were used as a measure of the antifilarial activity [32]. The aqueous extract of *T. crispa* was found to exhibit microfilaricidal activity which the microfilaricidal activity was investigated based upon direct observation of the microfilarial motility [33].

Antihyperglycaemic activity

The study of Noor and Ashcroft [34] indicated that the orally administrated extract of *T. crispa* exhibited significant antihyperglycaemic effect. The extract may consist of compounds which initiated the insulin secretion through the modulation of *β*-cell Ca^{2+} concentration. Thus, it can be further used as an antidiabetic agent for the treatment of type II diabetes.

Anti-nitric oxide (Anti-NO) activity

The aqueous extract of *T. crispa* was found to suppress nitric oxide oxide production by lipopolysaccharide-stimulated murine macrophages. Significant inhibitory activity against the NO level and inducible NO synthase was showed by ethyl acetate layer when partitioned with the aqueous extract. Both subfraction of E-3 and N-*trans*-feruloyltyramine displayed strong anti-NO activity [35,36]

Antioxidant activity

Based on DPPH, FRAP and TBA tests, the aqueous crude extract of *T. crispa* stem was found to exhibit high antioxidant activity and its antioxidative potency is comparable to the established antioxidants, such as BHT and vitamin C [37,38]. The antioxidant activity carried by Froemming [39] indicated that the methanol extract of *T. crispa* showed the highest antioxidant activity which was determined by measuring total flavonoid content, total phenolic content and DPPH free radical scavenging activity.

Antiparasitic activity

Rungruang & Boonmars [40] studied the *in vivo* antimalarial effect of the crude extract of *T. crispa* and the mice administrated of the extract with a dose of 80 mg/kg once a day were found to exhibit promising inhibitory activity against the parasite, *Plasmodium yoelii*.

Anti-proliferative activity

The result of anti-proliferative activity of the aqueous crude extract of *T. crispa* stem indicated a significant cytotoxic effect on certain human cancer cell lines such as MCF-7 (IC_{50}: 107 µg/mL), HeLa (IC_{50}: 165 µg/mL), Caov-3 (IC_{50}: 100 µg/mL) and HepG2 (IC_{50}: 165 µg/mL) [37] as compared to cisplatin and tamoxifen, the human anticancer drugs. Froemming [39] reported the methanolic extract of *T. crispa* displayed a dose-dependent cytotoxic effect on MDA-MB-231 and MCF-7 cancer cell lines with an IC_{50} value of 44.8 and 33.8 µg/mL, respectively.

Atherosclerosis inhibitory activity

The study conducted by Amom *et al* [41] revealed *T. crispa* stem aqueous extract delays the development of atherosclerosis by suppressing the total cholesterol, triglycerides, low density lipoprotein levels but showed a significant increased level of high density lipoprotein.

Thioacetamide-induced hepatotoxicity

Oral administration of ethanolic extract of *T. crispa* twice daily for 8 weeks at a dose of 100 mg/kg and 200 mg/kg was found to possess a significant effect on the thioacetamide-induced liver cirrhosis in rats [42].

Cardio-protective activity

The cardio-protective studies on the n-butanol extract of *T. crispa* demonstrated the presence of at least three different cardiovascular-active components which acted through (1) β_2-adrenergic receptors to cause a decrease in blood pressure, and β_1- and β_2-adrenergic receptors to cause an increase in heart rate, (2) α-adrenergic receptors to cause an increase in blood pressure and heart rate, and (3) a non-adrenergic and noncholinergic pathway to cause a decrease in MAP and heart rate [43].

Central analgesic activity

The dried extract of stem of *T. crispa* exhibited promising central analgesic activity at a dosage of 666 mL [44].

Cytochrome inhibitory activities

Usia *et al* [45] studied the inhibitory effects of 30 indonesian medicinal plants against cytochrome P450 3A4 (CYP3A4) and CYP2D6 with a radiometric assay and suggested that *T. crispa*, showed inhibitory activity over 70% on the metabolism mediated by CYP3A4. Subehan *et al* [46] showed that among 30 other plants in an inhibitory assay of cytochrome P450 3A4 (CYP3A4) and CYP2D6 via erythromycin *N*-demethylation and dextromethorphan *O*-demethylation activities in human liver microsomes that *T. crispa*, exhibited more than 30 % increase of CYP3A4 inhibition.

Hypoglycemic effects

Oral administration of *T. crispa* extract was found to display potent in vitro insulinotropic activity in the human and rat islets and HIT-T15 B cells [47]. Sriyapai *et al* [48] examined the *T. crispa* dry powder for hypoglycemic effect on the patients with metabolic syndrome. Administrated of 250 mg *T. crispa* dry powder twice a day for 2 months was proven to decrease fasting blood glucose significantly from the baseline. The study by Noipha and Ninla-aesong [49] indicated that the extract of *T. crispa* enhances the glucose uptake by in L6 myotubes which was associated to the increased levels of GLUT1 transporter, AMPKα and PPARγ transcript.

CONCLUSION

A large number of secondary metabolites and biological activities have been reported from *T. crispa*; however, it would be valuable to conduct bioassay-guided phytochemical studies on this plant in order to isolate new biologically important secondary metabolites.

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