Anti-inflammatory Activities of Extracts of *Cinnamomum porrectum* (Roxb.) Kosterm. Wood (Thep-tha-ro)

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Background: *Cinnamomum porrectum (Roxb.) Kosterm. (Thai name Thep-tha-ro) is a medicinal and aromatic tree mostly distributed throughout southern Thailand. It is traditionally used as spices, vegetable, carminative, tonic, febrifuge and postpartum care for being anti-inflammation whilst chemical components and its anti-inflammatory activity of this plant extracts were rarely reported.*
Objective: *To investigate the anti-inflammatory activity of the crude extracts from different extraction method of C. porrectum wood.*
Material and Method: *Steam distillation, maceration in 50% or 95% ethanol or decoction method were used for extraction. The extracts were obtained as oil, ethanolic or aqueous extracts, respectively. These extracts were tested in vitro for anti-inflammatory activity using nitric inhibitory assay by determining the inhibitory activity on lipopolysaccharide (LPS) induced nitric oxide production in RAW 264.7 cell lines.*
Results: *The 50%, 95% ethanolic extracts of this plant showed high anti-inflammatory activity (IC_{50} values as 19.28±2.43 and 13.78±3.76 μg/ml, respectively), where water extract of this plant showed low anti-inflammatory activity (IC_{50} values >100 μg/ml), oily extracts showed cytotoxicity.*
Conclusion: *The results revealed that ethanolic extracts showed strong anti-inflammatory activity; therefore these extracts of Cinnamomum porrectum should be further developed as a health product for treatment of inflammation.*

Keywords: *Anti-inflammatory activity, Cinnamomum porrectum, Thep-ta-ro, Nitric oxide, Ethanolic extracts*

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Cinnamomum porrectum (Roxb.) Kosterm. (common name Thep-tha-ro), family Lauraceae. is a native aromatic plant grown in Southeast Asia. This plant is mostly found in the southern part of Thailand and nominated as a provincial plant of Phang-nga. Its local names are Thep-tha-ro, Chuang, Chuang-hom, Cha-kai-hom, Phluu-ton-khaao and Mue-dae-ka-maa-nging. This plant was used as decorative sculpture and as goods from Trang province. Wood fragment was steam-distilled to obtain oil. Its wood is traditionally used as heart tonic, tonic for menstruation, anti-flatulent, to reduce colic, carminative, antipyretic, febrifuge, anti-insect attack, flavoring food, scent for soap, applied after childbirth and postpartum care as an anti-inflammatory[1]. However, there are a few reports on the extracts from different parts of the plant having antioxidant and antibacterial activities[2]. The wood of this plant is an ingredient in the Jatuwatapon remedy which was reported not to have any cytotoxic activity against breast cancer cells and low antioxidant activity[3]. However, there is no report on anti-inflammatory activity, yet we have done preliminary study on inhibitory effect on Nitric oxide release[4]. The results were interesting as the ethanolic extracts showed good inhibition on nitric oxide production. Thus, the objective of this research was to study the anti-inflammatory effect of its wood extracts to develop a drug and thus adding value to the wood fragments from the plant.

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Material and Method

Plant materials and extraction methods

The wood of *Cinnamomum porrectum* (Roxb.) Kosterm.was collected from Trang province, Thailand. Authentication of plant materials are carried out at the herbarium of the Department of Forestry, Bangkok, Thailand where the herbarium voucher specimen (Chuakul908/BK60803) is kept.

The wood was washed, sliced thinly, dried at 50°C and powdered. It was extracted by three different methods namely maceration, steam distillation and decoction methods.

Steam distillation

Steam distillation was used to obtain the oil extract. The plant material (500 g) was loaded into the extraction chamber filled with water, and heated to soften the plant fiber so that the oil molecules is released[5].

Maceration

Dried plant material (600 g) was macerated in 50% or 95% ethanol, each for 3 days, filtered and repeated 3 times where the filtrates were pooled and concentrated using an evaporator. Then, the dried residue from maceration in 50% or 95% ethanol or from steam distillation was further extracted by decoction.

Decoction

Dried plant material (500 g) was boiled in distilled water at boiling point for 30 min, filtered and dried by using freeze dryer to obtain water extract.

The percentage of yield from all extracts was calculated. The extracts were then kept in freezer (-20°C) before use.

Anti-inflammation by nitric oxide inhibitory assay

Cell culture

RAW 264.7 murine macrophage cell line was cultured in RPMI 1640 medium (Sigma, St. Luise, MO, USA) which supplemented with 10% heated fetal bovine serum (FBS) (Sigma, St. Louis, MO, USA), 100 U/ml penicillin and 100 µg/ml streptomycin and incubated at 37°C, 95% humidity in 5% CO_2 atmosphere. Cell line was subcultured every 3 days.

Measurement of nitric oxide (NO)

NO production was measured in cell culture supernatants of macrophages[6-8]. The murine macrophage cells (RAW 264.7) in RPMI-1640 medium supplement with 10% FBS and 1% P/S were seeded in 96-well plates with $1x10^5$ cells/well for 24 hour. Then cells were stimulated with 5 µg/ml lipopolysaccharide (LPS) in the presence of test samples at various concentration for 24 hours at 37°C in 5% CO_2. NO production was determined by measuring the accumulation of nitrite in the culture supernatant using the Griess reagent (0.1% naphthalene diaminedihydrochloride, 1% sulfanilamide in 5% H_2SO_4). Cytotoxicity was determined to confirm that nitric oxide productions were not occurred by destroying the cell membrane. This testing was performed using MTT assay[9] or the 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) colorimetric method. The absorbance was determined at 570 nm.

Statistical analysis

The results were expressed as mean$\pm$SEM of four determinations at each concentration, for each sample. The IC_{50} was calculated in percentage by the Prism program using the equation:

Inhibition (%) = (A-B) x 100/(A-C)

A-C: NO concentration (µM) [A: LPS (+), sample (-); B: LPS (+), sample (+); C: LPS (-), sample (-)].

Results

The effect of *Cinnamomum porrectum* (Roxb.) Kosterm. wood extracts on the inflammatory mediator (NO) in activated murine macrophage cell line (RAW 264.7) was measured as anti-inflammatory effect compared with positive control (indomethacin and prednisolone). The inhibitory activity against LPS induced NO production were shown in Table 1. The 50% and 95% ethanolic extracts of this plant each showed effective anti-inflammatory activity (IC_{50} values as 19.28$\pm$2.43, 13.78$\pm$3.76 µg/ml respectively). Whereas water extract of this plant showed low anti-inflammatory activity (IC_{50} values >100 µg/ml), the inhibitory effect on NO production of the two ethanolic extracts is more potent than indomethacin (IC_{50} = 92.76$\pm$2.01 µg/ml). Oily extract showed cytotoxicity against RAW246.7. However, all extracts were less potent than prednisolone (IC_{50} = 1.31$\pm$0.05 µg/ml) which is a positive control as an anti-inflammatory control drug. The residue of ethanolic extract, residue of water extract and oily extract had no anti-inflammatory activity.

Discussion

Inflammation is a part of the biological responses of the body against chemical, physical and pathogen stimuli to protect themself. NO is one of the

Table 1. Percentage of yield from *Cinnamomum porrectum* (Roxb.) Kosterm. extracts and the half maximal inhibitory concentration (IC_{50}) of inhibitory effect on NO production

Plant extracts	%Yield	% inhibition of NO production (% viability)						IC_{50}±SEM (µg/ml)
		0.1	1	10	30	50	100	
Oil	4.34	-	24.16±0.43 (71.48±1.26)	27.99±0.98 (58.50±4.16)	44.67±1.38 (57.67±1.71)	79.64±4.80 (55.77±2.58)	-	33.56±0.53
50% ethanol	3.03	-	22.36±0.24 (68.89±3.25)	37.71±3.15 (73.80±1.60)	60.90±1.28 (76.74±1.42)	92.69±0.33 (73.70±0.32)	-	19.28±2.43
95% ethanol	4.19	-	26.65±2.09 (89.64±3.22)	46.32±4.09 (80.02±4.81)	-	60.63±1.49 (73.70±0.32)	73.81±3.11 (69.99±3.43)	13.78±3.76
Water extract	0.02	-	-	-	-	-	-	>100
Decoction of residue after steam distillation	0.01	-	-	-	-	-	-	>100
Decoction of residue after 50% ethanol	0.32	-	-	-	-	-	-	>100
Decoction of residue after 95% ethanol	0.29	-	-	-	-	-	-	>100
Indomethacin	-	-	0.06±5.08 (94.85±1.53)	1.42±3.95 (99.16±1.76)	-	16.16±1.81 (83.98±3.41)	56.68±1.65 (85.21±2.02)	92.87±1.48
Prednisolone	-	32.35±1.87 (96.03±1.29)	46.11±0.48 (92.56±3.18)	58.45±1.17 (91.29±1.80)	-	64.86±2.03 (88.21±1.07)	74.03±1.17 (93.43±6.65)	1.31±0.05

inflammatory mediators causing inflammation. The 50% and 95% ethanolic extracts of *Cinnamomum porrectum* wood showed higher effective anti-inflammatory activity (IC_{50} values as 19.28±2.43, 13.78±3.76 µg/ml, respectively) than indomethacin (IC_{50} = 92.76±2.01 µg/ml). They showed anti-inflammatory activity 4.81 and 6.73 times higher than that of indomethacin but they are less potent compared with prednisolone (IC_{50} = 1.31±0.05 µg/ml), i.e. having IC_{50} of 14.71, 10.51 times lower, respectively. Water extract of this plant had no anti-inflammatory activity (IC_{50} values >100 µg/ml), and its oily extract showed cytotoxicity. The oil showed cytotoxic against RAW 246.7. It is concluded that 95% ethanolic extract showed the best anti-inflammatory activity. Thus, anti-inflammatory compound should be isolated from ethanolic extracts by bioassay guided isolation. Previously, there is no report of anti-inflammatory compounds of *Cinnamomum porrectum* (Roxb.) Kosterm. wood extracts obtained by different extraction methods and testing of its anti-inflammatory activity. Thus, this is the first report of in vitro testing of anti-inflammatory activity of this plant wood extracts. However, for further study on anti-inflammatory product development of this plant, the ethanolic extracts should be used. In addition, the 50% and 95% ethanolic extracts have been reported to have antioxidant activities as showing high DPPH radical scavenging activities (EC_{50} value as 19.26±0.47, 13.18±3.95 mg/ml respectively)[3], thus related with anti-inflammatory activities of its extracts in the present study as the inflammatory process induces oxidative stress and reduces cellular antioxidant capacity. Therefore, the high electron scavenging activity relates with good anti-inflammatory activity. It is also relevant that Thai traditional medicine used liquor or spirit as tonic or tonic for menstruation, antipyretic, febrifuge, and postpartum care as an anti-inflammatory[1]. Therefore, this plant was useful as an ingredient for the development of drugs for anti-inflammatory treatment, which can inhibit production of nitric oxide, one of the inflammatory mediators which may play an important role in the chronic inflammatory process. There is only one report of the methanolic extract of *Cinnamomum camphora* wood which showed antioxidant and anti-inflammation activities[11]; this previous result is supported by this study regarding the anti-inflammatory effect of the ethanolic extracts of *Cinnamomum porrectum* wood.

Conclusion

In summary, the ethanolic extract showed higher anti-inflammatory activities than the oily and the water extracts. This result supports the use of this plant in Thai traditional medicine which is in the form of liquor or spirit as a tonic for treatment of inflammatory-related diseases such as menstruation, as antipyretic, febrifuge, or applied after childbirth and as postpartum care. The crude ethanolic extract of *C. porrectum* should be developed as drugs for anti-inflammatory treatment. However, future investigation of *C. porrectum* should be on testing of anti-inflammatory activities through various pathways such as through TNF-α, PGE2 or cytokine.

What is already known on this topic?

Cinnamomum porrectum (Roxb.) Kosterm. (common name Thep-Tha-ro), family Lauraceae. Its local names are Thep-Tha-ro, Chuang, Chuang-hom, Cha-kai-hom, Phluu-ton-khaao and Mue-dae-kamaa-nging. It is traditionally used as heart tonic, tonic for menstruation, anti-flatulent, decrease colic, carminative, antipyretic, febrifuge, insect attack, flavoring food, scent for soap, applied after childbirth and postpartum care as an anti-inflammation. Many previous studies have shown that these aromatic plants were medicinally used and display inflammatory properties which protect the human body against cellular inflammation.

What this study add?

The present study not only showed the good inflammatory activity of 50% ethanol, 95% ethanol extracts of *Cinnamomum porrectum* (Roxb.) Kosterm., but also had studied the inflammatory activity of extracts obtained from other different methods of extraction such as oil, water, 50% or 95% ethanol, decoction of residue after macerated in 50% or 95% ethanol, decoction of residue from steam distillation compared with indomethacin and prednisolone. This results support the use of this plant wood in Thai traditional medicine where it was macerated and used in the form of liquor or spirit for treatment of inflammatory related diseases, i.e. as tonic for menstruation, antipyretic, febrifuge, treatment after childbirth or postpartum.

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Potential conflicts of interest

None.

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ฤทธิ์ยับยั้งการหลั่งไนตริกออกไซด์ของสารสกัดเทพธาโร

เพียงเพ็ญ ภักดีกำจร, ศรีโสภา เรืองหนู, อรุณพร อิฐรัตน์

ภูมิหลัง: *เทพธาโร (Cinnamomum porrectum (Roxb.) Kosterm.) เป็นพืชสมุนไพรมีกลิ่นหอมซึ่งพบมากทางใต้ของประเทศไทยภูมิปัญญาพื้นบ้านของไทยนิยมใช้เป็นเครื่องเทศ ผักจิ้มน้ำพริก ยาขับลม ยาบำรุง ยาแก้ไข้ และช่วยลดการอักเสบในมารดาหลังคลอด แต่ยังไม่มีรายงานฤทธิ์ที่เกี่ยวข้องกับการต้านการอักเสบ*

วัตถุประสงค์: *เพื่อศึกษาฤทธิ์ต้านการอักเสบของสารสกัดจากเนื้อไม้เทพธาโรด้วยวิธีการสกัดที่แตกต่างกัน*

วัสดุและวิธีการ: *สกัดด้วยการกลั่นไอน้ำหมักด้วยเอทานอล 50% หมักด้วยเอทานอล 95% หรือสกัดด้วยการต้มน้ำได้สารสกัดชั้นน้ำมัน ชั้นเอทานอลและชั้นน้ำตามลำดับ และกากที่ได้นำไปสกัดต่อด้วยการต้ม นำสารสกัดทั้งหมดไปทดสอบฤทธิ์ต้านการอักเสบ ด้วยวิธียับยั้งการหลั่งไนตริกออกไซด์จากเซล RAW 264.7 ที่ถูกกระตุ้นด้วย LPS*

ผลการศึกษา: *พบว่าสารสกัดชั้นเอทานอล 50%, เอทานอล 95% มีฤทธิ์ที่ดีในการต้านการอักเสบ (IC_{50} เท่ากับ 19.28±2.43 และ 13.78±3.76 μg/ml ตามลำดับ) ในขณะที่สารสกัดชั้นน้ำมีฤทธิ์ต้านการอักเสบต่ำ (IC_{50} >100 μg/ml) ส่วนสารสกัดชั้นน้ำมันมีความเป็นพิษต่อเซลล์*

สรุป: *จากผลการศึกษาพบว่าสารสกัดชั้นเอทานอลมีฤทธิ์ที่ดีในการต้านการอักเสบดังนั้นจึงควรนำมาพัฒนาเป็นผลิตภัณฑ์สำหรับลดการอักเสบต่อไป*