Inhibitory Effect on Nitric Oxide Production and Free Radical Scavenging Activity of Thai Medicinal Plants in Osteoarthritic Knee Treatment

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Background: *Thai medicine plants used for Osteoarthritis of knee (OA) treatment consist of twelve plants such as Crinum asiaticum, Cleome viscosa, Drypetes roxburghii, Piper longum, Piper nigrum, Plumbago indica, Alpinia galanga, Curcuma aromatica, Globba malaccensis, Zingiber montanum, Zingiber officinale and Zingiber zerumbet. They showed high frequency in OA formula.*
Objective: *To investigate inhibitory effect on LPS-induced nitric oxide (NO) release from RAW 264.7 cell and free radical scavenging activity using DPPH assay of these ethanolic plant extracts.*
Material and Method: *Plant materials were extracted by maceration in 95% ethanol. Anti-inflammatory activity were tested on LPS-induced NO production. Free radical scavenging activity was performed by DPPH assay.*
Results: *All of ethanolic extracts exhibited potent inhibitory effect on NO release. The ethanolic extract of Z. zerumbet exhibited the highest inhibitory effect followed by Z. montanum and G. malaccensis, respectively. Except A. galanga and C. viscosa, all extracts possessed more influential than indomethacin (IC_{50} = 20.32±3.23 µg/ml), a positive control. The investigation on antioxidant activity suggested that the ethanolic extracts of D. roxburghii, Z. officinale, Z. montanum, C. aromatic, A. galanga, P. indica, G. malaccensis, P. nigrum exhibited antioxidant activity. By means of D. roxburghii had the highest electron donating activity, followed by Z. officinale. Moreover, both extracts were more effective than BHT, a positive control (EC_{50} = 14.04±1.95 µg/ml).*
Conclusion: *Thai medicinal plants had anti-inflammatory activity and could inhibit destruction of articular cartilage that corresponded to the traditional medicine and supported using these medicinal plants for OA treatment.*

Keywords: *Osteoarthritis of knee, Thai medicinal plants, Inhibitory effect on nitric oxide release, Antioxidant activity*

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Osteoarthritis of knee (OA) is a chronic disease which frequently occurs in middle age and old people[1]. Chief complaint of this disease are cardinal signs of inflammation which lead to knee joint deformity and eventually crippling[2]. Nitric oxide (NO) is one of the inflammatory mediators that acts as important role in the inflammatory process and destruction of articular cartilage. The function of NO in the inflammatory process is increased vasodilation and vascular permeability, stimulating white blood cells to release interleukin-1 (IL-1) and a tumor necrosis factor (TNF). The role of NO in the destruction of articular cartilage are induced apoptosis, inhibited chondrocyte proliferation, inhibited synthesis of extracellular components including collagen and proteoglycan, inhibited IL-1 receptor antagonist synthetic, disturbed intrigrin action, stimulated metrix mettallo proteinases (MMPs) and inhibited tissue inhibitors of metallo proteinase (TIMP), which lead to increased articular cartilage destruction[3]. Previous studies in human indicated that nitrite accumulation in synovial fluid were increased in OA patients[4]. In addition, NO concentrations in synovial fluid of OA patients were higher than normal. It suggested that NO performs as a

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significant factor in pathogenesis of OA[5]. Furthermore, NO can react with superoxide anion to become peroxynitrite which has longer half life and reacts with protein result in tissue damage and TIMP inhibition[6].

Thai traditional medicine has long been used from various medicinal plant to treat OA for pain relief, swelling and knee joint arthritis. The first line of using Thai medicinal plants to treat OA with Thai traditional medicine[2] are twelve plants which have been used very frequently in many remedies such as *Crinum asiaticum* L, *Cleome viscosa* L, *Drypetes roxburghii* (Wall) Hurusawa, *Piper longum* L, *Piper nigrum* L, *Plumbago indica* L, *Alpinia galanga* (Linn) Swartz, *Curcuma aromatica* Salisb, *Globba malaccensis* Ridl, *Zingiber montanum* (Koenig) Link ex Dietr, *Zingiber officinale* Roscoe and *Zingiber zerumbet* (L) Sm.

Previous study of biological activities of some plants, which related with OA, produced no report. The investigation of biological activities in vitro is necessary to approve these medicinal plants in clinical use according to Thai traditional medicine. Hence, the objectives of this research were to investigate the inhibitory effects on LPS-induced NO release and antioxidant activities of these medicinal plants to clarify activity that has significance in the inflammatory process and cell defense mechanism against articular cartilage destruction.

Material and Method

Plant material

Plant materials were collected from several parts of Thailand in June 2011 (shown in Table 1) and

Table 1. The ethnobotanical data and biological activities of Thai medicinal plants

Scientific name	Thai name	Plant part	Place for specimens collection	Voucher specimen number	Biological activities
Crinum asiaticum AMARYLLIDACEAE	Plub plueng	Leaf	Suphanburi	SKP 008 03 01 01	Antiinflammation[18] Antioxidant[22]
Cleome viscosa1 CAPPARIDACEAE	Phug sian phi	Whole part	Nakornpathom	SKP 039-1 03 22 01	Antiinflammation[23] Antioxidant[24] Antipyretic activity[25] Analgesic[26]
Drypetes roxburghii EUPHORBIACEAE	Ma kum gai	Leaf	Kanjanaburi	SKP 071 04 18 01	n/a
Piper longum PIPERACEAE	Dee pli	Fruit	Supanburi	SKP 146 16 03 01	Antiinflammation[17,27] Antioxidant[27] Analgesic[28]
Piper nigrum PIPERACEAE	Prig thai lon	Seed	Juntaburi	SKP 146 16 14 01	Antiinflammation[29] Antioxidant[29]
Plumbago indica PLUBAGINACEAE	Jet ta mool Ploeng Dang	Root	Kanjanaburi	SKP 148 16 09 01	Antiinflammation[30] Antioxidant[30]
Alpinia galanga ZINGIBERACEAE	Kha	Rhizome	Uttaradit	SKP 206 01 07 01	Antiinflammation[19-21] Antioxidant[31]
Curcuma aromatica ZINGIBERACEAE	Van nang kum	Rhizome	Nakornpathom	SKP 206 03 01 01	n/a
Globba malaccensis ZINGIBERACEAE	Van ron thong	Rhizome	Nakornpathom	SKP 132 12 04 01	n/a
Zingiber montanum ZINGIBERACEAE	Plai	Rhizome	Kanjanaburi	SKP 206 26 03 01	Antiinflammation[12-14] Antioxidant[32] Antipyretic activity[12] Analgesic[12]
Zingiber officinale ZINGIBERACEAE	Khing	Rhizome	Nakornpathom	SKP 206 26 15 01	Antiinflammation[15] Antioxidant[15,16]
Zingiber zerumbet ZINGIBERACEAE	Kra tue	Rhizome	Kanjanaburi	SKP 206 26 26 01	Antiinflammation[10] Antinociception[33]

identified by Assoc. Prof. Dr. Arunporn Itharat. The voucher specimens were deposited at the herbarium of Southern Center of Thai Medicinal Plants at the Faculty of Pharmaceutical Science, Prince of Songkla University, Songkhla, Thailand.

Preparation of extracts

Powdered materials were macerated with 95% ethanol (EtOH) at room temperature (rt) for 3 days, filtered, and concentrated by evaporator. The residue was continuously macerated 2 times. Three crude extracts were combined together and calculated percentage of yield. Stock solutions (10 mg/ml) of the extracts were prepared in DMSO and stored at -20°C until use.

Cell culture

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

Measurement of nitrite

Nitrite production, an indicator of NO synthesis, was measured in the culture supernatant of macrophages, as described previously report[7]. Briefly, RAW 264.7 were seeded in 96-wells microplate with $1x10^5$ cells/well and incubated for 1 hour. The extracts were diluted as various concentration with complete media. The cells were stimulated with 5 µg/ml lipopolysaccharide (LPS) (Sigma, St. Louis, MO, USA) together with test samples and incubated for 48 hours. Indomethacin was used as a positive control. NO production was determined by measuring the accumulation of nitrite in the culture supernatant using Griess reagent (1% sulfanilamide and 0.1% naphthyl ethylene diaminedihydrochloride in 2.5% phosphoric acid). The absorbance was determined at 570 nm and calculated IC_{50} from percentage of inhibited NO production at various concentrations by prism program.

Determination of cell viability

Cell viability was also determined using a MTT assay[8]. After culture cells in a 96-well plate with LPS and test samples for 48 hours, the cells were incubated with 0.5 mg/ml MTT reagent (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) for 2 hours. The medium was discarded and 0.04M HCl in isopropanol was then added. Absorbance at 570 nm was determined by using microplate reader.

Scavenging effect on DPPH radical

The effect on DPPH (2,2-diphenyl-2-picrylhydrazyl) radical was estimated according to the method of Shimada et al[9]. Briefly, $6x10^{-5}$ M DPPH (100 µl) in absolute EtOH were added to samples solution (100 µl). The mixture was left to stand (rt.) without light for 30 minutes. Microplate reader at 520 nm measured the absorbance of the resulting solution. The EC_{50} values were calculated from percentage of free radical inhibition at various concentrations by Prism program. Butylatedhydroxytoluene (BHT) (FLUKA, Germany) was used as a reference compound.

Statistical analysis

The results were expressed as mean $\pm$ SEM of four determinations at each concentration for each sample. The IC_{50} values were calculated using the prism program.

Results

Inhibitory effect on NO production

Effect of Thai medicinal plant extracts on the pro-inflammatory mediator (NO) in activated murine macrophage cell line were measured as anti-inflammatory properties compared with positive control (indomethacin). The results of inhibitory activity against LPS induced NO production were shown in Table 2. The results indicated that all of the plant extracts possessed potent anti-inflammatory activity by inhibition of NO production. The ethanolic extract of *Z. zerumbet* showed the most effective, followed by *Z. montanum*, *G. malaccensis*, *P. nigrum*, *Z. officinale*, *P. indica*, *P. longum*, *C. asiaticum*, *D. roxburghii*, *C. aromatica*, *A. galanga* and *C. viscosa*, respectively (IC_{50} <30 µg/ml). Moreover, these plant extracts including *Z. zerumbet*, *Z. montanum*, *G. malaccensis*, *P. nigrum*, *Z. officinale*, *P. indica*, *P. longum*, *C. asiaticum*, *D. roxburghii* and *C. aromatica* exhibited inhibitory effect on NO production more potent than indomethacin (IC_{50} = 20.32 µg/ml) which is a positive anti-inflammatory drug.

Scavenging effect on DPPH radical

Electron donating activity, by means of 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, was investigated for the assessment of antioxidative activity. The results are shown in Table 3. The results suggested that several plant extracts

Table 2. Percentage of yield from Thai medicinal plant extracts, the half maximal inhibitory concentration (IC_{50}) of inhibitory effect on NO production

Plant extract	% yield	% inhibition of NO production (% viability)								IC_{50} ± SEM (µg/ml)
		0.01 µg/ml	0.1 µg/ml	1 µg/ml	5 µg/ml	10 µg/ml	30 µg/ml	50 µg/ml	100 µg/ml	
Z. zerumbet	4.48	5.78±0.01 (99.99±0.01)	11.21±0.01 (99.98±0.02)	15.83±0.00 (99.55±0.01)	69.50±0.01 (99.55±0.00)	73.37±0.01 (97.25±0.00)	-	-	-	4.05±0.00
Z. montanum	5.60	3.09±0.01 (99.78±0.01)	8.12±0.01 (95.81±0.01)	13.13±0.02 (85.23±0.00)	67.20±0.02 (72.03±0.01)	-	-	-	-	4.35±0.00
G. malaccensis	10.85	14.19±0.02 (99.93±0.01)	16.61±0.01 (98.16±0.01)	22.06±0.01 (98.00±0.02)	-	62.74±0.00 (80.36±0.00)	85.54±0.02 (70.72±0.01)	-	-	8.15±0.01
P. nigrum	5.64	1.46±0.04 (99.94±0.03)	2.76±0.02 (97.68±0.03)	9.72±0.02 (95.47±0.02)	32.21±0.21 (94.14±0.05)	54.32±0.02 (89.85±0.05)	-	-	-	8.91±0.02
Z. officinale	4.53	6.84±0.01 (99.93±0.01)	15.32±0.02 (98.25±0.01)	40.06±0.01 (98.23±0.01)	-	52.46±0.02 (84.23±0.01)	66.11±0.01 (77.47±0.02)	-	-	9.11±0.02
P. indica	9.17	1.02±0.01 (99.42±0.01)	7.12±0.02 (99.15±0.01)	8.44±0.01 (98.21±0.01)	10.49±0.00 (97.15±0.01)	51.78±0.00 (96.91±0.02)	-	-	-	9.74±0.00
P. longum	8.99	2.67±0.03 (87.53±0.04)	8.26±0.01 (83.60±0.01)	10.99±0.04 (76.00±0.01)	-	50.22±0.02 (74.98±0.04)	-	-	-	9.98±0.00
C. asiaticum	17.37	3.46±0.05 (94.94±0.00)	4.12±0.00 (83.48±0.01)	4.36±0.05 (76.61±0.01)	5.69±0.00 (72.23±0.01)	50.07±0.00 (71.38±0.00)	-	-	-	9.99±0.00
D. roxburghii	11.71	-	-	25.17±0.62 (96.81±9.79)	-	46.84±0.63 (89.20±15.05)	-	54.12±0.24 (90.55±18.62)	94.67±1.45 (86.16±11.70)	11.77±0.44
C. aromatica	6.22	4.25±0.02 (99.97±0.01)	13.34±0.02 (98.81±0.01)	23.33±0.01 (98.23±0.01)	-	27.70±0.01 (97.68±0.00)	68.42±0.02 (97.11±0.01)	-	-	12.27±0.01
A. galanga	5.74	-	4.97±0.01 (99.95±0.01)	14.85±0.01 (99.78±0.01)	-	46.18±0.01 (98.79±1.16)	-	70.63±0.02 (95.03±0.01)	-	21.50±0.09
C. viscosa	7.68	-	-	4.89±0.44 (96.09±23.85)	-	17.52±2.05 (87.75±11.17)	-	87.64±0.35 (94.05±6.71)	97.00±2.96 (79.08±11.28)	26.42±2.17
Indomethacin										20.32±3.23

Data represent Mean ± SEM of four independent experiments; "–" means "not done"

Table 3. The half maximal effective concentration (EC_{50}) of free radical scavenging activity and percentage of DPPH radical inhibition at various concentration

Plant extract	% inhibition at various concentration (µg/ml)				EC_{50} ± SEM (µg/ml)
	1	10	50	100	
D. roxburghii	7.33±2.93	59.63±9.83	95.99±0.73	96.48±2.14	8.29±1.50
Z. officinale	11.77±0.77	42.29±1.41	81.87±2.00	90.47±0.61	12.83±0.63
Z. montanum	9.09±0.42	24.79±1.35	71.21±1.27	82.19±0.60	27.39±1.35
C. aromatica	4.89±0.81	22.64±0.18	67.62±0.27	84.47±1.22	28.83±0.55
A. galanga	4.80±0.41	20.83±0.61	67.65±0.54	83.75±2.67	30.63±0.29
P. indica	3.47±1.55	20.51±0.41	57.51±1.62	79.98±3.27	36.66±0.92
G. malaccensis	6.14±3.87	13.08±1.80	37.57±3.31	61.91±1.46	74.58±5.16
P. nigrum	4.90±3.13	11.13±1.33	28.93±1.77	51.32±0.43	97.16±1.14
Z. zerumbet	-	-	-	-	>100
P. longum	-	-	-	-	>100
C. asiaticum	-	-	-	-	>100
C. viscosa	-	-	-	-	>100
BHT	8.77±0.37	39.74±3.88	77.92±6.17	88.13±0.52	14.04±1.95

Data represent mean ± SEM of four independent experiments; "–" means "not done"

Table 4. Inhibition of NO production and free radical scavenging activities of Thai medicinal plant extracts

Plant extracts	Inhibition of NO production IC_{50} ± SEM (µg/ml)	Free radical scavenging activity EC_{50} ± SEM (µg/ml)
Z. zerumbet	4.05±0.00	>100
Z. montanum	4.35±0.00	27.39±1.35
G. malaccensis	8.15±0.01	74.58±5.16
P. nigrum	8.91±0.02	97.16±1.14
Z. officinale	9.11±0.02	12.83±0.63
P. indica	9.74±0.00	36.66±0.92
P. longum	9.98±0.00	>100
C. asiaticum	9.99±0.00	>100
D. roxburghii	11.77±0.44	8.29±1.50
C. aromatica	12.27±0.01	28.83±0.55
A. galanga	21.50±0.09	30.63±0.29
C. viscosa	26.42±2.17	>100
Indomethacin	20.32±3.23	-
BHT	-	14.04±1.95

exhibited free radical scavenging activity. The ethanolic extracts which possessed potent electron donating activity were *D. roxburghii, Z. officinale, Z. montanum* and *C. aromatica*, respectively (IC_{50} <30 µg/ml). Plant extracts that had modulate/moderate electron donating activity were *A. galangal* and *P. indica* (IC_{50} = 30-50 µg/ml), *G. malaccensis* and *P. nigrum* (IC_{50} = 50-100 µg/ml), respectively. Plant extracts of *Z. zerumbet, P. longum, C. asiaticum* and *C. viscose* were devoid of antioxidative activity (IC_{50} >100 µg/ml). Furthermore, *D. roxburghii* and *Z. officinale* had the highest electron donating activity among all extracts and more effective than BHT as a positive control. These results suggested that *D. roxburghii* and *Z. officinale* extracts are composed of free radical scavenging compounds.

Inhibitory effect on NO production and scavenging effect on DPPH radical

The ethanolic extracts which possessed potent both inhibition of NO production and electron

donating activity were *Z. montanum*, *Z. officinale*, *D. roxburghii*, *C. aromatica* and *A. galanga*, respectively. Plant extracts that exhibited high activity in the inhibition of NO production, but had less electron donating activity, were *G. malaccensis*, *P. nigrum* and *P. indica*, respectively. Whereas, plant extracts including *Z. zerumbet*, *P. longum*, *C. asiaticum* and *C. viscosa* were active on inhibition of NO production, but showed no antioxidative activity.

Discussion

The study on both biological activities indicated that all of ethanolic extracts exhibited potent anti-inflammatory on inhibitory effect of NO production. Interestingly, all plant extracts except *A. galanga* and *C. viscosa* had an inhibitory effect of NO production and were higher than Indomethacin, a positive control. The anti-inflammatory effects of *Z. zerumbet* and *Z. cassumunar* were four fold and *G. malaccensis*, *P. nigrum*, *Z. officinale*, *P. indica*, *P. longum*, *C. asiaticum* were more than two fold higher than that of Indomethacin. It is suggested that these plant extracts contain compounds that have IC_{50} values considerably lower than that of Indomethacin. These results related with the previous study on active conpounds of *Z. zerumbet*[10,11], *Z. montanum*[12-14], *Z. officinale*[15,16] *P. longum*[17] *C. asiaticum*[18] and *A. galanga*[19-21]. Finding of these active compounds had been shown to inhibit significantly LPS-induced nitric oxide synthase (iNOS) expression and production of NO in macrophages and to block peroxynitrite-induced oxidation and nitration reactions in vitro in a dose-dependent manner but these reports did not exhibit the results on their ethanolic extracts. These substances are useful as lead compounds for the development of a novel class of anti-inflammatory agents, which can inhibit production of NO, one of the inflammatory mediators that play an important role in the inflammatory process and destruction of artricular cartilage. Whereas, previous study on biological activity of *P. indica* indicated that root ethanolic extract exhibited moderate inhibitory effect on LPS-induced NO release from RAW 264.7 and weak on electron donating activity[20]. It may result from quality control of a crude drug which was the most important effect contributing to its activity. Furthermore, the results of *G. malaccensis*, *D. roxburghii*, *C. aromatica* and *C. viscosa* on inhibitory effect of NO production, which possessed potent activity, had never been reported.

The results of antioxidant activity in present study suggested that five ethanolic extract including *D. roxburghii*, *Z. officinale*, *Z. montanum*, *C. aromatica* and *A. galanga* also possessed the potent electron scavenging that related with defense mechanism of the reaction of nitrite with superoxide anion.

Conclusion

These results corresponded with the Thai traditional medicine and supported using these Thai medicinal plants for OA treatment. Further investigation of anti-inflammatory activity on various path ways are necessary to continue, for choosing plant extract containing active compounds that possess potent anti-inflammatory agents on related pathways.

What is already known on this topic?

Osteoarthritis of knee is chronic disease which cardinal signs of inflammation and leads to knee joint deformity and crippling. Many Thai medicinal plants are used in the treatment of osteoarthritis of knee in middle age and old people. However, these plants are the subject of little scientific reporting in support of using them to treat osteoarthritis of knee. Thus, these results can support using these twelve plants that were used to treat OA.

What this study adds?

The present study investigated anti-inflammatory activity and antioxidant activity of twelve medicinal plants by iNOS and ROS, which is oxidative stress for chronic diseases including inflammation of OA. All ethanolic extracts showed an inhibitory effect on NO production release and ethanolic extract of *D. roxburghii*, *Z. officinale*, *Z. montanum*, *C. aromatica* and *A. galangal* also exhibited antioxidant activity. These results supported using these Thai medicinal plants for OA treatment.

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Potential conflicts of interest

None.

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ฤทธิ์ยับยั้งการหลั่งไนตริกออกไซด์และฤทธิ์ต้านอนุมูลอิสระของสมุนไพรไทยที่ใช้รักษาโรคข้อเข่าเสื่อม

อารียา อนุเฉกิงกุล, อรุณพร อิฐรัตน์

ภูมิหลัง: *ศาสตร์การแพทย์แผนไทยมีสมุนไพร 12 ชนิด ที่มีความนิยมและมีความถี่สูงในตำรับยาที่นำมาใช้รักษาโรคข้อเข่าเสื่อม ได้แก่ พลับพลึง, ผักเสี้ยนผี, มะคำไก่, ดีปลี, พริกไทย, เจตมูลเพลิงแดง, ข่า, ว่านนางคำ, ว่านร่อนทอง, ไพล, ขิง และกระทือ*

วัตถุประสงค์: *เพื่อศึกษาฤทธิ์ต้านการอักเสบโดยการยับยั้งการหลั่งไนตริกออกไซด์และฤทธิ์ต้านอนุมูลอิสระด้วยวิธี DPPH scavenging assay ของสารสกัดสมุนไพรด้วยเอทานอล 95%*

วัสดุและวิธีการ: *ตัวอย่างพืชถูกนำไปสกัดด้วยวิธีการแช่สกัดด้วยแอลกอฮอลทดสอบฤทธิ์ต้านการอักเสบโดยศึกษาฤทธิ์ยับยั้งการหลั่งไนตริกออกไซด์ที่ถูกกระตุ้นด้วย LPS ฤทธิ์ต้านอนุมูลอิสระศึกษาโดยวิธี DPPH scavenging assay*

ผลการศึกษา: *สารสกัดของสมุนไพรทุกชนิดมีฤทธิ์ดีในการยับยั้งการหลั่งไนตริกออกไซด์ โดยที่สารสกัดสมุนไพรทุกชนิด ยกเว้นข่าและผักเสี้ยนผีมีฤทธิ์ดีกว่าสารมาตรฐานอินโดเมทาซิน (Indomethacin) (IC_{50} = 20.3±3.23 ไมโครกรัม/มิลลิลิตร) การทดสอบฤทธิ์ต้านอนุมูลอิสระพบว่า สารสกัดมะคำไก่, ขิง, ไพล, ว่านนางคำ, ข่า, เจตมูลเพลิงแดง, ว่านร่อนทอง และพริกไทย มีฤทธิ์ยับยั้งอนุมูลอิสระ โดยที่สารสกัดมะคำไก่และขิงมีฤทธิ์ดีที่สุด ซึ่งดีกว่าบีเอชที (BHT) ที่ใช้เป็นสารมาตรฐาน (EC_{50} = 14.04±1.95 ไมโครกรัม/มิลลิลิตร)*

สรุป: *สมุนไพรที่ใช้รักษาโรคข้อเข่าเสื่อมมีฤทธิ์ในการต้านการอักเสบ และยับยั้งการถูกทำลายของเซลล์กระดูกอ่อนผิวข้อ ซึ่งสอดคล้องกับการนำมาใช้ในทางการแพทย์แผนไทย*