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Analgesic, anti-inflammatory and antiemetic activities of *Cleome scaposa* DC.

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Abstract

Cleome scaposa DC., has long been used in traditional herbal medicine for the treatment of pain and inflammation. The present investigation is an attempt to evaluate antiemetic, analgesic and anti-inflammatory activities of *Cleome scaposa* leaves methanolic extract by using chick emesis model (oral treatment), analgesy meter test (intraperitoneal treatment) in rats and carrageenan induced rat paw edema (oral treatment) respectively. The antiemetic activity (150 mg/kg b.w., of extract) was carried out by using chlopromamize (150mg/kg) as standard antiemetic drug. The analgesic activity (250 mg/kg b.w., of extract) was performed by using diclofenac sodium (50mg/kg) as standard analgesic drug whereas, anti-inflammatory activity (500mg/kg b.w., of extract) was done and indomethacin (10mg/kg) was taken as standard anti-inflammatory drug. The results showed significant antiemetic, analgesic and anti-inflammatory effects.

Keywords: *Cleome scaposa* ; antiemetic; analgesic; anti-inflammatory

Introduction

Plant derived substances are continue to have a place in the process of drug discovery, especially in the development of new analgesic and anti-inflammatory drugs. They not only help in the development of new analgesic and anti-inflammatory drugs but also greatly contributed to understand complex pathway of pain transmission, receptor types and endogenous ligands involved in pain transmission. Isolation of morphine (*Papver somniferum*), tetrahydrocannabinol (*Cannabis sativa*), Capsaicin (*Capsicum* species), Salicylic acid (*Salix* species) prove the involvement of opioid (μ,δ and κ), cannabinoid (CB_1 and CB_2), vanilloid receptors and cycloooxygenase enzyme respectively in pain and inflammation. The search for new naturally occurring analgesic and anti-inflammatory compounds is intensifying because of their effectiveness, lack of serious side effects and providing significant leads in the development of more effective synthetic molecules (Calixto *etal.*,2000). Search for analgesic and anti-inflammatory secondary metabolites proved alkaloids, flavonoids, steroids and terpenoids as analgesic (Calixto *etal.*,2000) whereas alkaloids, fatty acids, polyphenolics (fla-

vonoids, lignans, phloroglucinols, quinines, phenylpropanoids, stilbenes and diarylheptanoids), steroids, terpenoids (Gautam & Jachak,2009; Agnihotri *etal.*,2010), saponins and polysaccharides (Agnihotri *etal.*,2010) behave as anti-inflammatory agents. Similarly, diarylheptanoids (Yang *et al.,* 1999c,d ; Shin *et al.*,2002; Yang *etal.,* 2002), flavonoids (Kinoshita *et al.,* 1996; Yang *et al.* 1999a,b,d; Shin *et al.*,2002), glucosides (Yang *et al.,* 1999b), lignans (Kinoshi-ta *et al.*,1996; Yang *etal.,* 1999b), monoterpenes (Kinoshita *et al.*,1996), sesquiterpenes (Kinoshi-ta *etal.*,1996; Yang *et al.,* 1999d), triterpenes (Kinoshita *et al.*,1996; Eda *et al.*,2005), phenylpropanoids (Kawai *et al.*,1994; Kinoshita *etal.*,1996), polysaccharides (Maki *et al.,* 1987) and sterols (Yang *etal.,* 1999; Shin *etal.*,2002) are reported as active anti-emetic principles.

Cleome scaposa DC., (Family: Capparaceae) is found as a common weed all over the plains of Arabia, Egypt, India, Pakistan and throughout the tropics of the world (Ali, 1973). It is an annual, 10-30 cm tall, Leaves simple. Flowers 3-4 mm across, actinomorphic, white turning yellowish rarely pinkish; Capsule linear, 20-30 mm long. Seeds about 0.6 mm in diam., brown-black. *Cleome* species have been used in different abdominal complaints (Atiqur *etal.,* 2004; Khan, 2009) and given in pain and inflammation (Qureshi *etal.,* 2010). Trinortriterpenoid dilactone (brachycarpone), deacetoxybrachycarpone, cabralealactone, ursolic acid are reported from *Cleome* species (Viqaruddin & Khisal, 1987).The chemical structure of these compounds are mention in Figure 1. *Cleome scaposa* DC., has been used traditionally as analgesic, antipyretic and anti-inflammatory agent (Hameed *et al.,* 2011).

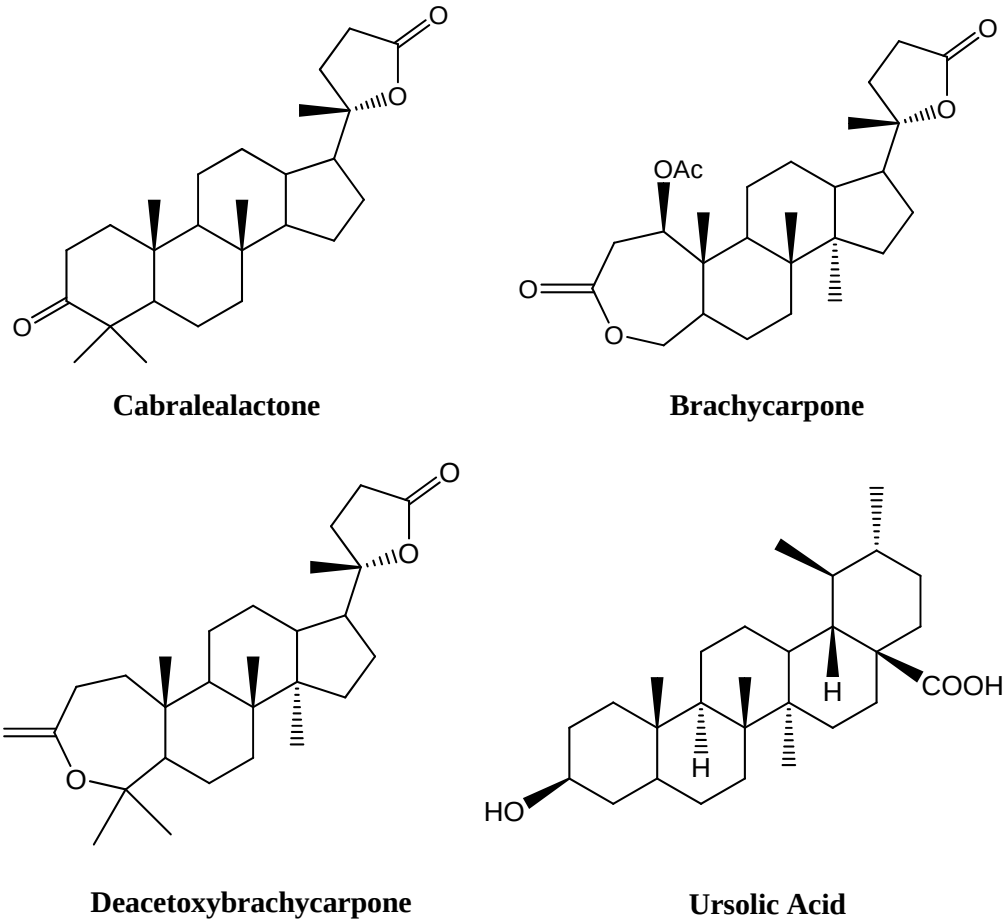

Figure 1. Chemical structure of constituents isolated from Cleome species.

Materials and Methods

Plant material

Leaves of *Cleome scaposa* DC., were collected by Mr. Arshad Gohar from University of Karachi in July 2011 identified by a taxonomist and a voucher specimen (G.H.No. 86450) was deposited in the herbarium of Department of Botany, University of Karachi.

Extraction

Leaves were dried in shade at room temperature for 7 days then soaked in methanol for 5 days at room temperature and filtered through filter paper. Methanolic extract was obtained by using rotary evaporator under reduce pressure at 40^{o}C.

Animals

Young male chicks, 4 days old (32-52 g) and Whistar albino rats (150-200g) of both sexes were obtained from Big-bird Poultry Breeders (Pvt) Ltd., and Animal house of Aga Khan University, Karachi, Pakistan respectively. They were housed in plastic cages with saw dust as beddings under temperature 25 ± 2°C; 12 h/12 h light-dark cycle and given food and water *ad libitum*. The animals were treated complying with the international standards for dealing the experimental animals duly approved by the legal bodies of the University of Karachi. Chicks (for antiemetic activity) and rats (for analgesic and anti-inflammatory activities) were randomly divided into three groups. The groups of animals were transferred in different cages and marked with their identification. Permission and approval from animal studies were obtained from Board of Advanced Studies and Research, University of Karachi [BASR. Res. No.25 (15)-2007].

Chemicals used

Copper (II) sulfate pentahydrate (copper sulfate) was purchased from Scharlau Chemie S.A. Barcelona, Spain. 3-(2-chloro-10*H*-phenothiazin-10-yl)-*N*,*N*-dimethyl-propan-1-amine (chlorpromazine) was purchased from ICN, USA. DMSO, Tween 80 and methanol were purchased from Merck, Darmstadt, Germany. Indomethacin), Carrageenan and Diclofenac sodium were purchased from Sigma-Aldrich Corporation.

Analgesic activity

Analgesic activity was determined by using Analgesy meter test (Randall & Selitto , 1957). The group of rats were treated intraperitoneally with normal saline, *Cleome scaposa* DC., leaves extract (250mg/kg each) and standard (diclofenac sodium 50mg/kg). The left hind paw of rat was placed on a plinth under a cone-shaped pusher of the Ugo Basile analgesia meter (No. 7200). It generates a linearly increasing mechanical force or pressure on hind paw. As the applied pressure increases, it gets to a point where the animal struggles to free its paw. The strength at which each rat withdrew its paw was recorded and considered as indicative of pain. The reaction strength of each rat was determined before and at 1, 2 and 3hrs after treatment with standard drug or plant extract. Stimulus was terminated and force thres-

hold read in grams taken as soon as nociceptive response was elicited by the rats. Inhibition of pain (%) or pain threshold was calculated as follows:

Pain threshold = (Treated mean – Control mean/ Control mean) x 100.

Anti-Inflammatory activity

The anti-inflammatory activity of methanol extract of *Cleome scaposa* DC., leaves (500 mg/kg) was evaluated following the protocols of Winter and co-workers (1962) with slight modifications (Rimbau *etal.,* 1996). Oedema was induced by subplantar injection of carrageenan (0.1 ml of 1% solution in 0.9% saline solution) into the left hind paws. Paw volume was measured after 3rd hour of carrageenan treatment by means of volume displacement methods using the Ugo Basile plethysmometer (No.7140). The difference between initial and after treatment paw volumes indicated the degree of inflammation. Oedema was expressed as a percent increase in paw volume due to carrageenan administration referred to the noninjected paw. The average increase in paw volume of each group was calculated and compared with the control (saline) and the indomethacin (10 mg/kg orally) groups. Oedema inhibitory activity was calculated according to the following formula:

% reduction of edema = (C - T / C) x 100

Where: C = % swelling of control group (untreated) and T = % swelling of treated group.

Antiemetic activity

The anti emetic activity was evaluated by using chick emesis model (Akita *etal.,* 1998). Each chick was placed in a large beaker and left to settle for 10 minutes. The methanol extract of *Cleome scaposa* DC., leaves was prepared as a dose of 150 mg/kg body weight in a volume of 10 ml/kg in 0.9% saline containing 5% DMSO and 1% Tween 80. The dose was administered abdominally. The control group received only saline 0.9%. After 10 minutes, copper sulfate was administered orally at 50 mg/kg b.w., and the number of retches was observed during the next 10 minutes. Chlorpromazine was used as a standard antiemetic drug (150 mg/kg body weight). The percent inhibition was calculated by the following formula:

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

Where, A = Frequency of retching in control group; and B = Frequency of retching in test group

Statistical analysis

Antiemetic, analgesic and anti-inflammatory activities were expressed as mean ± standard error of mean. The statistical significance of the difference was determined by an unpaired Student's *t*-test.

Table 1. Analgesic activity of *Cleome scaposa* leaves extract.

Groups	Pain threshold ± S.E.M (% inhibition of pain)				% Inhibition average
	Before Drug administration	After Drug Administration			
		1 hr	2 hr	3 hr	
Control	36.66±1.7	39.16±2.3	40.83±2.7	35.3±2.3	---
DS 50 mg/kg i.p.	53.3±2.0	81.34±2.7 * (26.86)	102.2±2.3** (40.40)	70.4±3.2** (16.27)	27.84
CS 250 mg/kg i.p.	45.8±2.7	57.9±2.3** (32.36)	82.5±5.5** (50.50)	70±4.9** (49.57)	44.14

DS = Diclofenac sodium , CS= *Cleome scaposa* , i.p. = intraperitoneal treatment, Values are mean ± SEM, N=6 for each group, **P*<0.05 &***P*<0.005 vs. control showing significant and most significant values using unpaired Student's *t*-test.

Results and Discussion

The intraperitoneal administration of *Cleome scaposa* DC., leaves extract (250 mg/kg) showed significant antinociceptive effect against mechanical pain (Table 1).The analgesia-meter test is a useful method in elucidating centrally mediated antinociceptive responses. The crude extract increased nociceptive threshold of rat further strengthening the evidence of centrally mediated antinociceptive activity (Nkeh et al., 2002). The methanol extract of *Cleome scaposa* at the dose 250 mg/kg,i.p., significantly reduced the animal sensitivity to pain induced by pressure. The % average inhibition from pain was found to be 27.84 of diclofenac sodium and 44.14 of *Cleome scaposa.* The central protecting effect of *Cleome scaposa* leaves extract was comparable to diclofenac sodium. In this model of pain, *Cleome scaposa* leaves extract significantly increased the ability of animals to withstand pressure-induced pain indicating a more central acting mechanism of the extract.

At the dose of 500 mg/kg the *Cleome scaposa* DC leaves extract showed maximum inhibition of the oedema (69.16 %) as compared to control. The oedema inhibition by Indomethacin 10 mg/kg p.o. was tested as 44.67% (Table 2). Oedema development in carrageenan-induced paw oedema model in rats is represented by two phases (Vinegar *et al.,* 1969). The first phase occurs within an hour of carrageenan injection and is partly due to the trauma of injection and also due to release of histamine and serotonin (Crunkhon & Meacock, 1971). Oedema induced by carrageenan after 3rd hour of injection indicates the second phase of applying acute inflammatory model which is mediated by prostaglandins, the cycloxygenase products and lipoxygenase products (Vinegar *et al.,* 1969). Non-steroidal anti-inflammatory agents inhibit cyclooxygenase (COX-2) enzymes involved in prostaglandin synthesis (Robinson, 1997; Kulkarni *et al.,* 2000). Based on these reports it is possible that the inhibitory effect of the *Cleome scaposa* DC leaves extract on carrageenan-induced inflammation in rats could be due to inhibition of cyclooxygenase leading to inhibition of prostaglandin synthesis. Although the cyclooxygenase and lipoxygenase pathways are both involved in the inflamm-

Table 2. Anti-inflammatory activity of *Cleome scaposa* leaves extract.

Groups	Mean paw oedema (ml) ± S.E.M at 3rd hour	% inhibition of inflammation
Control	78.8±9.7	------
Std. (IND) 10 mg/kg p.o.	43.6±1.7	44.67*
CS 500 mg/kg p.o.	24.3±0.3	69.16*

IND= Indomethacin, CS= C*leome scaposa*, N = 6 for each group, p.o. = per oral, S.E.M.= Standard Error of Mean,* *P*<0.01vs. control showing significant values using unpaired Student's *t*-test.

Table 3. Antiemetic activity of *Cleome scaposa* leaves extract.

Groups	Mean number of Retches±S.E.M	Inhibition (%) of emesis
Control	68.12±3.88	-----
Std. (CPZ) 150 mg/kg p.o.	45.0±0.28*	33.94
CS 150 mg/kg p.o.	34.1±2.16**	49.94

CPZ= Chlorpromazine, CS= C*leome scaposa*, N = 6 for each group, p.o.= per oral, S.E.M.= Standard Error of Mean, **P* <0.1 and ***P*<0.005 vs. control showing significant and most significant values values using unpaired Student's *t*-test.

atory process, inhibitors of cyclooxygenase are more effective in inhibiting carrageenan-induced inflammation than lipoxygenase inhibitors (Flower *et al.,* 1980). In our experiment, rats pre-treated with *Cleome scaposa* DC leaves extract showed a significant oedema inhibitory response after 3rd hour following carrageenan injection. This result suggests that *Cleome scaposa* DC leaves extract may act by suppressing the later phase of the inflammatory process by the inhibition of cyclooxygenase. So, inhibition of carrageenan induced paw oedema by the crude extract could be due to its inhibitory activity on the prostaglandins.

Cleome scaposa DC., leaves extract in dose of 150 mg/kg body weight reduced the numbers of retches by 49.94% (Table 3). The group of chicks treated with chlorpromazine had 45 retches compared to the 68 retches of the control group, thus chlorpromazine reduced the retches by 33.94%. From the results it is clear that the extract has antiemetic potential and is comparable with standard chlorpromazine. Although the result is significant but the mode of action is not known. However, as the oral copper sulphate induces emesis by peripheral action and peripheral 5- HT_4 plays an important role in copper sulfate induced emesis (Bhandari *et al.,* 1991; Fukui *et al.,* 1994), the extract was able to effectively prevent its effect, it could be implied that the extract has a peripheral antiemetic action.

As mentioned earlier that triterpenoids such as brachycarpone, deacetoxybrachycarpone, cabralealactone and ursolic acid are reported from *Cleome* species (Viqaruddin & Khisal, 1987). Triterpenoids possess analgesic (Biswas *etal.,*2009) anti-inflammatory (Singh *etal.,*2006; Gautam & Jachak,2009) and antiemetic (Kinoshita *etal.,*1996) properties. Analgesic and anti-inflammatory activities of ursolic acid also has been proven (Vasconcelosa *etal.,* 2006).So, if triterpenoids are present in the studied extract it may be implied that the observed analgesic, anti-inflammatory and antiemetic activities may be due to the presence of these triterpenoids.

It may be said that *Cleome scaposa* DC., leaves extract has analgesic, anti-iflammatory and antiemetic effects. These results support the traditional use of C*leome scaposa* in some painful conditions and gastro intestinal complaints. Further investigations are required to establish responsible analgesic, anti-inflammatory and antiemetic compound(s) and elucidate exact mechanism of action.

Conflict of interest

There is no conflict of interest associated with the authors of this paper.

References

Winter CA, Risley EA, Nuss GW. (1962). Carrageenan induced edema in the hind paw of rat as an assay for anti-inflammatory activity. *Proceedings of the Society for Experimental Biology and Medicine* 111, 544–547.

Agnihotri S,Wakode S, Agnihotri A.(2010). An overview on anti-inflammatory properties and chemo-profiles of plants used in traditional medicine. *Indian Journal of Natural Products and Resources* 1(2):150-167.

Akita Y, Yang Y, Kawai T, Kinoshita K, Koyama K, Takahashi K, Wantanabe K. (1998). New assay method for surveying anti-emetic compounds from natural sources. **Natural** *Product Sciences* 4:72-77.

Ali SI. (1973). Capparidaceae. In: Nasir E and Ali SI, editors. Flora of Pakistan. Volume 34, Fakhri Printing Press, Karachi, pp 20-24,30.

Atiqur RM, Mossa JS, Al-Said MS, Al-Yahya MA. (2004). Medicinal plant diversity in the flora of Saudi Arabia 1: a report on seven plant families. *Fitoterapia* 75:149–161.

Bhandari P, Andrews PLR. (1991). Preliminary evidence for the involvement of the putative 5-HT4 receptor in zacopride- and copper sulfate-induced vomiting in the ferret. *European Journal of Pharmacology* 204, 273–280.

Biswas M, Biswas K, Ghosh AK, Haldar PK.(2009). A pentacyclic triterpenoid possessing analgesic activity from the fruits of *Dregea volubilis*. *Pharmacognosy Magazine* 5(19):90-92.

Calixto JB, Beirith A, Ferreira J, Santos ARS, Filho VC, Yunes RA. (2000). Naturally Occurring Antinociceptive Substances from Plants. *Phytotherapy Research* 14: 401–418.

Crunkhorn P, Meacock SCR**. (**1971). Mediators of the inflammation induced in the rat paw by carrageenan. *British Journal of Pharmacology* 42, 392-402.

Eda M, Hayashi Y, Kinoshita K, Koyama K, Takahashi K, Akutu, K.(2005). Anti-emetic Principles of Water Extract of Brazilian *Propolis*. *Pharmaceutical Biology,* 43(2): 184-188.

Flower RJ, Moncada S, Vane JR.(1980). Gilman AG Goodman LS and Gilman A (Eds), The pharmalogical basic of therapeutics, 6th ed., Macmillan & Co. New York, pp. 701-705.

Fukui H, Yamamoto M, Sasaki S, Sato S. (1994). Possible involvement of peripheral 5-HT4 receptors in copper sulfate-induced vomiting in dogs. *European Journal of Pharmacology* 257, 47– 52.

Gautam R, Jachak SM. (2009). Recent Developments in Anti- Infammatory Natural Products. *Medicinal Research Reviews* 29(5): 767—820. DOI 10.1002/med.20156

Hameed M, Ashraf M, Al-Quriany F, Nawaz T, Sajid M, Ahmad A, Younis A, Naz N.(2011), Medicinal Flora of the Cholistan Desert: A Review. *Pakistan Journal of Botany* 43(SI): 39-50.

Khan FM. (2009). Ethno-Veterinary Medicinal Usage of Flora of Greater Cholistan Desert (Pakistan). *Pakistan Veterinary Journal* 29(2): 75-80.

Kinoshita K, Kawai T, Imaizumi T, Akita Y, Koyama K, Takahashi K. (1996). Anti emetic principles of *Inula linariaefolia* flowers and *Forsythia suspensa* fruits. *Phytomedicine* 3: 51-58.

Kulkarni SK, Jain NK, Singh A. (2000) . Cyclooxygenase isoenzymes and newer therapeutic potential for selective COX-2 inhibitors. *Methods and Findings in Experimental and Clinical Pharmacology* 22: 291- 298.

Kawai T, Kinoshita K, Koyama K, Takahashi K. (1994).Anti-Emetic Principles of *Magnolia obovata* Bark and *Zingiber officinale* Rhizome. *Planta Medica* 60(1): 17-20.

Maki T, Takahashi K, Shibata S. (1987).An antiemetic principle of *Pinella ternata* tuber. *Planta medica* 53:410-414.

Nkeh C-AB, Njamen D, Wandji J, Fomum Z, Dongmo A, Nguelefack TB, Wansi B, Kamanyi A. (2003). Anti inflammatory and analgesic effect of Drypermolundein A, a sesquiterpene lactone from *Drypetes molunduana. Pharmaceutical Biology* 41(1): 26-30.

Qureshi R, Bhatti GR, Memon RA, (2010). Ethnomedicinal uses of herbs from Northern Part of Nara Desert, Pakistan. *Pakistan Journal of Botany* 42(2): 839-851.

Randall LO, Selitto JJ. (1957). A Method for Measurement of Analgesic Activity on Inflamed Tissue. *Archives Internationales de Pharmacodynamie et de Thérapie* CXI, No. 4: 409-419.

Robinson DR. (1997). Regulation of prostaglandin synthesis by antiinflammatory drugs. *Journal of Rheumatology Supplement* 47: 32-39.

Rimbau V, Risco E, Canigueral S, Iglesias J. (1996). Antiinflammatory Activity of Some Extracts from Plants used in the Traditional Medicine of North-African Countries. *Phytotherapy Research* 10:421-423.

Shin D, Kinoshita K, Koyama K, K Takahashi.(2002). Antiemetic Principles of *Alpinia officinarum*. *Jounal of Natural Product.* 65: 1315-1318.

Singh B, Sahu PM, Lohiya RK, Sharma MK, Singh HL, Singh S.(2006). Anti-inflammatory activity of alkanoids and triterpenoids from *Trichodesma amplexicaule* Roth. Phytomedicine 13(3):152–156.

Vasconcelosa MAL, Royoa VA, Ferreiraa DS, Crottia AEM, Andrade e Silvaa ML, Carvalhob J´CT, Bastosc JK, Cunha WR.(2006). *In vivo* Analgesic and Anti-Inflammatory Activities of Ursolic Acid and Oleanoic Acid from *Miconia albicans* (Melastomataceae). *Zeitschrift für Naturforschung* 61c:477-482.

Vinegar R, Schriber W, Hugo R. (1969). Biphasic development of carrageenan edema in rats. *Journal of Pharmacology and Experimental Therapeutics* 166; 96- 103.

Viqaruddin A, Khisal AA.(1987). Deacetoxybrachycarpone, a triterpenoid from *Cleome brachycarpa*. *Phytochemistry* 26(1): 315-316.

Winter CA, Risley EA, Nuss GW.(1962). Carrageenan-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. *Proceedings of the Society for Experimental Biology and Medicine* 111: 544-547.

Yang Y, Kinoshita K, Koyama K, Takahashi K, Tai T, Nunoura' Y, K.Watanabe. (1999a).Anti-emetic principles of *Pogostemon cablin* (Blanco) Benth. *Phytomedicine* 6(2): 89-93.

Yang Y, Kinoshita K, Koyama K, Takahashi K, Tai T, Nunoura' Y, K.Watanabe. (1999b).Novel experimental model using free radical-induced emesis for surveying anti-emetic compounds from natural sources. *Planta medica* 65:574-576.

Yang Y, Kinoshita K, Koyama K, Takahashi K, Tai T, Nunoura Y, Watanabe K. (1999c). Two Novel Anti-emetic Principles of *Alpinia katsumadai. Jounal of Natural Product* 62: 1672-1674

Yang Y, Kinoshita K, Koyama K, Takahashi K, Tai T, Nunoura Y, Watanabe K. (1999d). Anti-emetic principles of *Alpinia katsumadai* Hayata. *Natural Product Sciences* 5(1): 20–24.

Yang Y, Kinoshita K, Koyama K, Takahashi K, Kondo S, Watanabe K.(2002).Structure antiemeticactivity of some diarylheptanoids and their analogues. *Phytomedicine* 9(2):146–152.

