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Antinociceptive and anti-inflammatory activities of *Pandanus fascicularis* Lamk. leaves in animal models

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SUMMERY

The present study was carried out to elucidate the potential of, chloroform extract of *Pandanus (P.) fascicularis* Lamk (Family-Pandanaceae) leaves on antinociceptive, behavioral study and anti-inflammatory effects using various animal models The dried, powdered leaves of, *P. fascicularis* were extracted successively with petroleum ether (60 - 80°C) and chloroform in soxhlet apparatus. The chloroform extract (yield 21.6% w/w with respected to dry powdered plant material) was selected for all experimental procedure. Two models were employed to investigate the effects on nociception, the tail immersion and hot plate method in Swiss albino mice and anti inflammatory effect were investigated by employing the carrageenan induced rat paw edema test in. adult Wister albino rats. Behavioral study was investigated by elevated plus maze method in Swiss albino mice. Results were revealed that the PFCE was found significant antinociceptive effect ($P < 0.001$) at the dose levels of 100, 200 and 400 mg/kg, orally in mice and produced remarkable anti-inflammatory effect ($P < 0.001$) at the same dose levels used in the rats. Behavioral study of the PFCE has no significant anxiolysis effect when used orally. It concludes that, PFCE possessed remarkable antinociceptive effect and anti-inflammatory effect but no anxiolytic effect on animal models.

Key words: *Pandanus fascicularis* Lamk; Leaves; Chloroform extract; Antinociceptive; Anti-inflammatory; Behavioral study

INTRODUCTION

Pandanus (P.) fascicularis Lamk (Family-Pandanaceae) is a dioecious shrub, densely branched with copious aerial roots are found in the coastal region of India and Andaman Islands. (Anonymous, 1956) It is sufficiently found in the Coastal South Orissa of India. The plant is well known under vernaculars as '*Fragrant screw pine*' in English, '*Kia*' in Oriya, and '*Ketki*' in Hindi and '*Dhuli puspika*' in Sanskrit. (Anonymous, 1999; Chatterjee and Pakrashi, 2001; Nadkarni, 2002) The powdered leaves are useful in tumors, leprosy, antispasmodic and rheumatoid arthritis (Chopra, 1958; Vaidyaratnam, 1997; Chatterjee and Pakrashi, 2001). This plant contains main chemical constituents viz; cirsilineol, n–triacontanol, physcion, compesterol, daucosterol, β-sitosterol, β-sitostenone, stigmasterol and stigmust-4-en-3, 6-dione.(Rastogi and Mehrotra, 1995; Chatterjee and Pakrashi, 2001). The literature reveals that the *P. fascicularis* leaves are used orally against pain, inflammation and epilepsy in traditional system and most of the phytoconstitutes were isolated from leaves of *P. fascicularis*. Hence, the leaves of this plant have been used for all pharmacological activities. Due to the limitations

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of opioid and NSAID therapy, there is a necessary to continuing search for new analgesics and on account of the alleged usefulness of this plant in the traditional treatment of some painful and inflammatory conditions which has not yet been scientifically proved. Hence, an effort has been made to establish the scientific validity to investigate the possible antinociceptive, anti-inflammatory and also behavioral study of the crude chloroform extract of *P. fascicularis* leaves in animal models.

MATERIALS AND METHODS

Plant material

Pandanus fascicularis Lamk leaves were collected during the month of August from the rural belt of Arjipoli in Ganjam District, Orissa, India, identified and authenticated by Prof. S. K. Dash, HOD, PG Department of Bioscience, College of Pharmaceutical Sciences, Mohuda; comparing with the voucher specimen (PFL-I) present in the herbarium, has been kept in the laboratory for future references. The collected plants were washed and air-dried under the shade, cut into small pieces, powdered by a mechanical grinder and passed through 40-mesh sieve and stored in a closed vessel for future use.

Preparation of *Pandanus fascicularis* chloroform extract

The dried, powdered leaves of, *Pandanus fascicularis* Lamk (1 kg) were extracted successively with 1,200 ml of petroleum ether (60 – 80°C) and 1200ml of chloroform in soxhlet apparatus. A dark greenish black coloured petroleum ether extract was obtained. The same powdered leaves (marc), after proper drying, were extracted with chloroform (18 h) to produce a greenish brown semisolid mass. The extractions were carried out until the solvents became colourless. These extracts were again dried and concentrated by evaporating the solvent completely under vacuum at the range of boiling points of solvent (Chloroform at 62°C) using rotatory evaporator (Jain Scientific glass works, DTC 201, Ambala cantt, India). The chloroform extract (yield 21.6% w/w with respected to dry powdered plant material) was selected for all experimental procedure. The chemical constituents of the extract was identified by qualitative analysis and confirmed by the thin layer chromatography (i.e. hR_f values) PFCE was prepared an emulsion by triturating the accurately weighed quantity of the extract with 0.025% w/v of carboxyl methyl cellulose (CMC) used for the study. All extractive solvents are of analytical grade reagents (AR) and purchased from S.D. Fine Chemicals, Mumbai, India.

Preparation of drugs

Tramadol (Contramal, Nicholas Piramal India Limited, Mumbai.) was dissolved in 0.025% w/v of CMC. Diclofenac sodium (Diclomax, Torrent Pharmaceutical Pvt. Ltd., Ahmedabad, India) and carrageenan (Sigma Chemicals Company. St. Louis, MO, USA) were used for the Anti-inflammatory study. The standard drug diazepam (Calmpose, Ranbaxy Lab, India) was used for behavioral study. PFCE and standard drugs were prepared by suspending them in 0.025% w/v CMC at definite concentrations separately for all pharmacological studies.

Preliminary phytochemical analysis

The PFCE was subjected to preliminary phytochemical screening for detection of major chemical groups. In each case test 10% w/v solution of the extract in chloroform was used and unless otherwise mentioned in individual test (Patil, 2001).

Experimental animals

Adult wister albino rats weighing between 180 and 220 g and Swiss albino mice of either sex between 18 and 22 g were used for the experiments, obtained from M/s Ghosh & Ghosh Enterprises., Kolkata India, were housed in standard polypropylene cages at room temperature of 30 ± 2°C and 60 - 65% relative humidity and had free access to food and water *ad libitum*. The animals

were used for the experiment after an acclimatization period of one week. All procedures described were reviewed and approved by the University Animals Ethical Committee (Reference Code: 990/C/CPCSEA/ 2006), University Department of Pharmaceutical Sciences, (UDPS) Bhubaneswar, Orissa.

Acute toxicity analysis

Toxicity study of the PFCE was performed to get the information, how safe is this extract for the therapeutic use. The LD_{50} value of PFCE was derived by the method of Litchfield and Wilcoxon 1949. The maximum non-lethal dose was found to be 4,000 mg/kg body weight, orally. The 0.025% CMC was used as a vehicle and showed no mortality. The determination of acute toxicity by adopting fixed dose the guideline of CPCSEA and $1/10^{th}$ of LD_{50} cut off values (Patil, 2001; Shivakumar, 2007) of the extracts were taken as screening dose. i.e. 100, 200, 400 mg/kg for subsequent studies.

Antinociceptive activity

Antinociceptive activity of the PFCE was tested using the Experimental models of Tail immersion method and Hot plate method. In the tail immersion method, the tail of mouse was immersed to a constant level (5 cm) in a water bath maintained at 55 ± 0.5ºC. The time to flick the tail from water (reaction time) was recorded. A maximum immersion time of 30 s. was maintained to prevent thermal injury to the animals (Janssen *et al*., 1963). The reaction time was measured 30 min before test and reference standard. A significant increase in reaction time compared with control was considered a positive analgesic response. The Hot plate test was carried out using an UGO Basile hot plate apparatus (Socrel model D-S37, Italy). The hot plate test was used to measure latency time by the method of Hosseinzadeh *et al.* (2000). The temperature of the hot plate was maintained at 55 ± 0.5ºC to asses the thermal-induced antinociceptive activity as described by Turner 1965, Animals were placed into Perspex cylinder on the heated surface and the time between placement and licking of the paws or jumping was recorded as response latency. After 16 h. fasted mice were divided into five groups of six mice in each. Group-I, served as a control, received 0.025% w/v CMC, 10 ml/kg, orally, Group-II to IV, animals received PFCE at dose of 100, 200 and 400 mg/kg, orally and Group-V, animals were treated with tramadol (50 mg/kg, orally) as a positive-control. Cut off time for the response was set at 60 s to avoid tissue damage to the mice paws. (Junping, 2005) After the determination of baseline response latencies, hot-plate latencies were re-determined at 30min, 60min, and 90min after oral administration of tested drugs and positive-control in this experiment. The pain inhibition percentage was calculated (Wu *et al.,* 2003; Owoyele, 2004) according to the following formula. % of (PIP) = Latency (test) - Latency (control) ×100)/Latency (control).

Anti-inflammatory assay

Anti-inflammatory activity was evaluated using the carrageenan-induced edema in rat paw according to the technique of Winter *et al*., 1962 and Satyanarayana *et al*., 2004. After 16 h fasted rats were divided into four groups of six each. Group-I, served as a control, received 0.025% w/v CMC at the dose level of 10 ml/kg, orally, Group-II to IV, animals received PFCE at dose of 100, 200 and 400 mg/kg, orally, Group-V, animals were treated with standard drug diclofenac sodium at the dose level of 10mg/kg, orally. Acute inflammation was induced by carrageenan in sub planter side of the right hind paw in rats. The paw was marked with ink at the level of the lateral malleolus and dipped in Perpex cell up to this mark. The measurement of the paw volume was carried out by means of Ugo Basile Plethysmograph model 7150, before and after 4 h after carrageenan injection. (Harris and Spenser, 1962).Percentage inhibition of edema was calculated using formula (Mohammed and Kumar, 2005). % Pain Inhibition = (1- Vt/Vc) × 100. Where, Vt = Increase in paw volume in drug treated rats. Vc = Increase in paw volume in control group treated rats.

Behavioral analysis

Behavioral analysis of the animals was evaluated by Elevated plus maze method (EPM).The EPM apparatus consisted of two open arms (30 × 5 cm) and two closed arms (30 × 5 × 20 cm) emanating from a common central platform (5 × 5 cm). The two pairs of identical arms were opposite to each other. The entire apparatus was elevated to a height of 50 cm above the floor level. The animals received the treatment as per the schedule. 15 min. before the start of session. At the beginning of the session, a mouse was placed at the centre of the maze, its head facing the closed arm and allowed to explore the maze for 5 min. The time spent in the open arm, percent entries in the open and closed arms and total entries were recorded. An entry was defined as the presence of all four paws in the arm. (Guaraldo *et al.,* 2000) The EPM was carefully wiped with 10% ethanol after each trail to eliminate the possible bias due to the odor of the previous animals (Lister, 1987). Group-I, served as control, received 0.025% w/v CMC, 10ml/kg, orally, Group-II to IV, animals received PFCE at dose of 100, 200 and 400 mg/kg and Group-IV, animals were treated with standard drug diazepam at the dose level of 4 mg/kg, orally and the average time spent in both open and closed arm in each group of the mice were recorded.

Statistical analysis

The results were presented as Mean ± S.E.M. and statistical significance ($P < 0.001$) between treated and a control group was evaluated by paired *t*-test (Woodson, 1986).

RESULTS

Preliminary phytochemical analysis

Results of different chemical tests on the chloroform extract of *P. fascicularis* showed the presence of phytoconstituents viz., steroids, terpenoids, flavonoids, saponins and tannins.

Antinociceptive activity

The effects of the PFCE were used to investigate the antinociceptive effects in animal models by adopting two methods of tail immersion test and hot plate test are shown in Tables 1 & 2 respectively. The extract produced about 66 and 74% of PIP in test animals in case of tail immersion method at the dose of 200 and 400 mg/kg after every one-hour intervals. The results were found to be statistically significant ($P < 0.001$) antinociceptive effects and were comparable to the standard drug tramadol, which showed 70% of PIP at the dose of 50 mg/kg ($P < 0.001$). The centrally acting analgesics generally elevate the pain threshold of mice towards heat. PFCE significantly ($P < 0.001$) increased the reaction

Table 1. Antinociceptive activity of *Pandanus fascicularis* by tail immersion method

Groups	Dose (mg/kg)	Pretreatment	Post treatment			
			1 h	2 h	3 h	4 h
(Group-I) Control (0.025% CMC)	10 ml	1.6 ± 1.05	1.6 ± 1.12	1.6 ± 1.12	1.6 ± 1.12	1.6 ± 1.12
(Group-II) PFCE	100 mg /kg	1.6 ± 1.51	1.61 ± 1.01 (6.21%)	1.84 ± 1.0 (13.04%)	1.86 ± 1.0 (13.97%)	4.2 ± 1.02 (61.90%)
(Group-III) PFCE	200 mg/kg	1.8 ± 0.8	4.8 ± 0.9[a] (66.66%)	6.4 ± 1.5[a] (71.87%)	6.5 ± 1.21[a] (75.38%)	5.4 ± 1.12[a] (70.37%)
(Group-IV) PFCE	400 mg/kg	1.9 ± 1.01	6.2 ± 1.05 (74.19%)	6.5 ± 1.12[a] (75.38%)	6.3 ± 1.1[a] (74.60%)	7.0 ± 1.21[a] (77.14%)
(Group-V) Tramadol + control (Positive control)	50 mg /kg	1.8 ± 1.2	5.4 ± 1.12 (70.37%)	10.8 ± 1.0 (83.33%)	12 ± 1.0 (85.00%)	14.2 ± 1.01 (88.73%)

Results expressed as mean ± S.E.M. [a]$P < 0.001$ significantly different from control; Paired *t*-test (n = 6). Figures in the parentheses indicate % of (PIP) in mice.

Table 2. Antinociceptive activity of *Pandanus fascicularis* leaves by hot plate method

Treatment	Dose (mg/kg)	Paw licking time in seconds				Paw Jumping time in seconds			
		0	30	60	90	0	30	60	90
(Group-I) Control (0.025% CMC)	10 ml	3.0 ± 0.1	2.8 ± 0.3	2.6 ± 0.21	2.8 ± 0.1	3.0 ± 1.0	2.8 ± 0.8	2.7 ± 0.6	2.7 ± 0.4
(Group-II) PFCE	100 mg/kg	3.6 ± 0.31	3.6 ± 0.2[b] (22.22%)	4.6 ± 0.1[b] (42.22%)	5.0 ± 0.12[b] (44.44%)	3.6 ± 0.3	6.2 ± 0.1[b] (54.83%)	6.6 ± 0.2[b] (59.09%)	6.6 ± 0.4[b] (59.09%)
(Group-III) PFCE	200 mg/kg	3.6 ± 0.12	4.7 ± 0.15 (40.42%)	4.5 ± 0.1[b] (45.83%)	5.6 ± 0.12[b] (50.00%)	3.8 ± 0.3	6.7 ± 0.4[b] (58.20%)	6.6 ± 0.5[b] (59.09%)	7.6 ± 0.6[a] (60.29%)
(Group-IV) PFCE	400 mg/kg	3.8 ± 0.12	4.6 ± 0.2 (39.13%)	4.8 ± 0.8 (48.00%)	5 ± 0.5[a] (44.00%)	4.0 ± 0.6	5.8 ± 0.7 (51.72%)	5.8 ± 0.12 (53.44%)	6.8 ± 0.5[a] (64.47%)
(Group-V) Control + Tramadol	50 mg/kg.	3.4 ± 0.2	4.6 ± 0.3 (39.13%)	5.6 ± 0.12[a] (53.57%)	6.00 ± 0.15[a] (53.33%)	3.4 ± 0.1	6.6 ± 0.3[a] (57.57%)	7.6 ± 0.8[a] (64.47%)	8.2 ± 0.3[a] (67.07%)

Results expressed as mean ± S.E.M. [b]$P < 0.05$, [a]$P < 0.001$, significantly different from control; Paired *t*-test (n = 6). Figures in the parentheses indicate % of (PIP) in mice.

time of animals towards the thermal source in a dose-dependent manner. In hot plate test PFCE showed a pain inhibition percentage (PIP) of 60.29% and 64.47%, respectively whereas tramadol showed a greater PIP of 67.07% at 90 min after treatment.

Anti-inflammatory activity

Indigenous drug systems can be a source of variety of new drugs, which can provide relief in inflammation but their claimed reputation has to be verified on scientific basis. The present investigation revealed that the anti-inflammatory activity of *P. fascicularis* on carrageenan induced paw edema in rats is shown in Table 3. These results indicate that, PFCE showed significant reduction ($P < 0.001$) in edema volume at oral dose of 100, 200 and 400 mg/kg of body weight, which is comparable to the standard drug diclofenac sodium at the dose of 10 mg/kg in acute inflammatory model.

Behavioral study by EPM

In EPM, the behaviour, which was absorbed that, confirmed the anxiolytic activity of diazepam as reported. The effect of the PFCE on behavioral study by EPM in mice was depicted in Table 4. It administration of diazepam 4 mg/kg, i.p. dose produce significant anxiolytic effect indicated by increase in the open arm entries, time spent in open arm. However, there was no significant anxiolysis effect or impairment in behavioral of the animals observed with PFCE at the dose levels of 100, 200 and 400 mg/kg of body weight when administered orally.

Table 3. Anti-inflammatory activity of *Pandanus fascicularis* leaves on carrageenan induced paw edema in albino rats

Treatment	Dose (mg/kg)	Percentage of inhibition of paw edema after carrageen injection			
		1 h	2 h	3 h	4 h
(Group-I) Control (0.025% CMC)	10 mg/kg)	13.20 ± 1.98	35.31 ± 4.06	41.21 ± 4.06	41.03 ± 5.16
(Group-II) PFCE	100 mg/kg	30.92 ± 2.78[a]	70.82 ± 9.62[a]	100.72 ± 8.69[b]	110.54 ± 10.38[b]
(Group-III) PFCE	200 mg/kg	24.70 ± 4.20[a]	46.32 ± 1.01[a]	70.65 ± 7.80[a]	80.04 ± 4.56[a]
(Group-III) PFCE	400 mg/kg	19.68 ± 0.705[a]	40.03 ± 0.70[a]	62.9 ± 7.605[a]	69.783 ± 4.25[a]
(Group-V) Diclofenac sodium	10 mg/ kg	44.32 ± 2.45[a]	99.79 ± 3.63[a]	130.02 ± 2.53[a]	134.70 ± 5.35[a]

Results expressed as mean ± S.E.M. [b]$P < 0.01$, [a] $P < 0.001$, significantly different from control; Paired *t*-test (n = 6).

Table 4. Behavioral study of *Pandanus fascicularis* leaves by elevated plus maze (EPM) in mice

Treatment	Dose	Number of arm entry		Time spend in arms (seconds)	
		open	closed	open	closed
(Group-I) Control (0.025% CMC)	10 ml	2.2 ± 2.1	4.4 ± 2.4	48.4 ± 1.98	251.6 ± 1.91
(Group-II) PFCE	100 mg/kg	0.8 ± 1.15	1.4 ± 1.98	24.0 ± 2.13	228 ± 1.99
(Group-III) PFCE	200 mg/kg	1.6 ± 2.0	2.0 ± 2.18	24.0 ± 2.28	276 ± 2.018
(Group-III) PFCE	400 mg/kg	1.5 ± 2.18	2.0 ± 2.17	27.0 ± 2.01	274.5 ± 2.12
(Group-V) Control + Diazepam	4 mg/kg i.p.	5.8 ± 2.15	1.2 ± 2.01	268 ± 1.89	31.0 ± 1.98

Administration of the diazepam 4 mg/kg, i.p. dose produce significant anxiolytic effect compared to that of the control group. However, there was no significant anxiolysis effect observed in PFCE when administered orally.

DISCUSSION

In acute toxicity study, oral administration of PFCE did not produce any mortality in mice upto a dose level of 4 g/kg. This may be due to broad non-toxic range of the plant, where the plant extract showed a high LD_{50} and relatively safety. The antinociceptic effect of PFCE was investigated by two well-established assay procedures. The antinociceptic action of all the tested compounds was clearly evident by a dose dependent reduction on tail immersion test and hot plate test are shown in Tables 1 & 2 respectively. These methods for investigating antinociceptive were selected such that centrally mediated effects were investigated. Evan though the present day armamentarium is rich in potent analgesic agents, the search for novel and safe analgesic drugs continues and vigorously pursued in many parts of world. The reasons are very obvious; the most potent opiate group of analgesics is associated with many undesirable side effects and also carries a potential for drug addiction. The other prominent groups of analgesics viz. NSAID are notorious for their ulcerogenic (Robert *et al*., 2001) and nephrotoxic potential (Consucio *et al*., 2005).In this regard, it is interesting to note that many flavonoids isolated from various plants exhibited potent analgesics and anti-inflammatory action. (Brasseur, 1989; Ferrandiz, 1991; Bllesteros *et al*., 1995; Sudheesh *et al*., 1997) It is also believed that those flavonoids ability to influence the said activities occur through modulation of the pro-inflammatory gene expression, such as inducible NO synthase and cyclooxygenase-2 (Dawson and Snyder, 1994). Due to these valid reasons, the plant *P. fascicularis* was explored for its antinociceptive and anti-inflammatory activities. The PFCE at the doses of 100, 200 and 400 mg/kg, p.o. tested was shown to possess antinociceptive activity in tail immersion method. It has been assumed that thermally motivated and tonic tests elicit the selective stimulation of Aδ and C fibers, respectively (Yeomans, 1996), it is tempting to propose that PFCE or its metabolites may interfere with the transmission of both fibers or with a common pathway, such as spinal and thalamic pathways. The hot-plate test was selected to investigate central analgesic activity, because it had several advantages, particularly the sensitivity to strong analgesics and limited tissue damage. Hence,the hot plate method was employed to verify if the extract could show any central analgesic effect, as the test is specifies analgesic test (Sulaiman *et al*., 2004). It was demonstrated that the PFCE at dose of 100, 200 and 400 mg/kg, p.o. widely used acute inflammatory model for studying anti-inflammatory agent The PFCE were found to be statistically significant ($P < 0.001$) antinociceptive effects and were comparable to the standard drug tramadol at the dose of 50 mg/kg. Edema represents the early phase of inflammation in carrageenan induced paw edema and is the simplest and most widely used acute inflammatory model for studying anti-inflammatory agent. The development of

carrageenan-induced edema is believed to be biphasic of which the first phase is mediated by release of histamine, serotonin and kinine in the first hour after injection of carrageenan and the second phase is related to release of prostaglandin like substances in 2 - 3 h. (Vinegar *et al*., 1969; Di Rosa *et al*., 1971; Brooks, 1991). The PFCE showed significant anti-inflammatory activity at 4 h against carrageenan injection suggesting that the extract predominantly inhibits the release of prostaglandin like substances from phlogenic stimuli. There are reports that flavonoid possesses anti-inflammatory activity (Ferrandiz, 1991; Ballesteros *et al*., 1995; Sudheesh *et al*., 1997; Jadhav, 2005) and some of them also act as phoshpholipase inhibitors. (Fowzy *et al*., 1988; Mikayw *et al*, 1993; Aitchdrfoun *et al*., 1996) Also, there are few reports on the experimental models, the non selective antagonist of opiod receptors apparently acts by antagonizing the action of endogenous opiods involved in pain or stress (Faden, 1998). In the present study, the maximum anti-inflammatory effect of PFCE may be attributed to presence of flavonoids as evident by preliminary phytochemical investigations. From the results it could be concluded that the extracts exhibit anti-nociceptive activity by central as well as peripheral mechanism(s). The behavioral study of the animals was evaluated by EPM. The EPM test is based on a premise where the exposure to an EPM evoked an approach avoidance conflict that was considerably stronger than that evoked by the exposure to an enclosed arm (Murugesan *et al*., 1999; Guaraldo *et al*., 2000). The decrease in aversion to the open arm is the result of an anxiolytic effect expressed by the increase time spent and entries in the open arm. Most of the sedatives and hypnotics drugs were implied by the method of EPM. Generally sedatives and hypnotics suppress cerebral activity. They also depress the CNS beginning with the cerebral cortex and descending with increasing dose to the medullury centers causing medullary paralysis (Grollman and Grollman, 1970). It was reported that the administration of diazepam 4 mg/kg, i.p. dose produce significant anxiolytic effect (Rabbani *et al*., 2003) indicated by increase in the open arm entries, time spent in open arm and closed arm. The control group and the dose levels of 100, 200 and 400 mg/kg of body weight of PFCE was not produce significant anxiolysis effect when compare to standard drug. Finally, it concluded that the PFCE possess remarkable antinociceptive, anti-inflammatory activity but no anxiolytic activity. However, more detailed phytochemical studies are necessary to identify the active principles and exact mechanisms of action.

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