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Analgesic and antiinflammatory activity of *Alstonia macrophylla* and *Mallotus peltatus* leaf extracts: Two popular ethnomedicines of Onge, a Negrito tribe of little Andaman

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SUMMARY

Two popular ethnomedicines of the Onge, a Negrito tribe of Andaman Islands, were evaluated for analgesic and antiinflammatory activity. The methanol extract as well as the different fractions of methanol extract of both *Alstonia macrophylla* and *Mallotus peltatus* leaves were studied using Swiss albino mice and Wistar albino rats. Acetic acid induced writhing, Tail flick and Tail immersion; Carrageenin- and Dextran-induced paw oedema tests were used. Dose-dependent analgesic and antiinflammatory activity were demonstrated for both methanol leaf extracts as well as fractions. Results were highly comparable with that of the standard drug pethidine.

Key words: *Alstonia macrophylla; Mallotus peltatus;* Triterpenoids; Sterols; Analgesic activity; Antiinflammatory activity

INTRODUCTION

The Andaman and Nicobar Island, situated about 1200 km off the eastern coast of mainland India on Bay of Bengal, is the homeland of 6 primitive aboriginal tribal groups, namely Jarawas, Sentineles, Onges, Great Andamanese, Great Nicobarese and Shompens. These people are surviving and sustaining good health for centuries without the help of modern medicines. On the other hand, the island is one of the richest tropical biodiversity zones of the world; contain about 2500 angiospermic species of which 14% are endemic. Several ethnobotanical surveys indicated that nearly 220, including 45 endemic, plant species are used as medicaments in the traditional health care system of the tribes and local people (Bhargava, 1983; Chakraborty and Vasudeva Rao, 1988; Dagar and Dagar, 1991). In an effort of screening tribal ethnomedicines for bioactive compounds, especially antimicrobials, we have studied two popular ethnomedicinal plants, *Alstonia macrophylla* and *Mallotus peltatus*, widely used by the Onge, an oldest seminomadic Negrito population of Dugong Creek of Little Andaman Islands, Great Andamanese and Nicobarese. Compared to other hostile tribes like Jarawas and Sentineles, the Onge are friendly, living in a small island in mid sea, and have there own primary health care system. *Alstonia macrophylla* Wall ex A. DC. (Apocynaceae), known as Chuharoi by the Onge, is a panatropical tree native to

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Malaysia and stretching to the Bay Islands. While *Mallotus peltatus* (Geist) Muell. Arg. var *acuminatus* (Euphorbiaceae), known as Pataque and Obottacke by the Onge, is a shrub endemic to the Little Andamans, Chidiyatappu, Baratang, Jarawa Creek and Interview Islands of South and Middle Andamans. Ethnobotanical reports indicate that the decoction of *A. macrophylla* leaves (AML) and stem bark is widely used to treat stomachache (Dagar and Dagar, 1991), skin diseases and urinary infections (Bhargava, 1983) among the Onges. The decoction of stem bark has anticholeretic and vulnerary effect (Asolkar *et al.*, 1992), while the leaf paste with hot coconut oil is used as a poultice to treat sprains, bruises and dislocated joints (Wealth of India, 1985) and also as febrifuge (Ambasta, 1992). Ethnobotanical literature reveals that the decoctions of *M. peltatus* leaves (MPL) and stem bark is used to treat stomachache (Dagar and Dagar, 1991), intestinal ailments, skin infections (Bhargava, 1983), while the alcoholic extracts of leaves are helpful in treating trematodic infections (Asolkar *et al.*, 1992). The recent work by this group showed that the AML extracts have moderate antibacterial, limited antifungal and significant anti-inflammatory activity (Chattopadhyay *et al.*, 2001; Arunachalam *et al.*, 2002). The MPL extract, on the otherhand, is found to have antibacterial (Chattopadhyay *et al.*, 2002a), and significant antipyretic activity (Chattopadhyay *et al.*, 2002b). The aim of the present study is to evaluate, for the first time, the analgesic as well as antiinflammatory activity of the methanolic extracts and the *n*-butanol fractions of methanol extract of *A. macrophylla* and *M. peltatus* leaves.

MATERIALS AND METHODS

Plant material

The leaves of *Alstonia macrophylla* Wall ex A. DC (Apocynaceae) and *Mallotus peltatus* (Geist) Muell. Arg. var *acuminatus* (Euphorbiaceae) were collected with the help of Onge native healers from the rain forests of North, Middle and South Andamans during April, June and October 1999 as well as in May, July and November 2001 to record the seasonal variation in the amount of active principle, if any. The voucher specimens have been identified (Herbarium No. 9220, 9221, 9227 and 9228 respectively) and deposited at the Herbarium Section of the Botanical Survey of India, Andaman & Nicobar Circle, Port Blair, India.

Extraction and fractionation

The dried leaves of *Alstonia macrophylla* and *Mallotus peltatus* were separately powdered (1 kg each) and successively extracted with 95% methanol for 72 h at room temperature. The whole extract was filtered and the solvents were evaporated to dryness under reduced pressure in an Eyela Rotary Evaporator (Japan) at 40 - 45°C. The percentage yield of the prepared extract was 8.9 ± 0.21 for AML and 8.7 ± 2.1 for MPL respectively. The preliminary phytochemical tests indicated the presence of tannins, triterpenoids, flavonoids, alkaloids, steroids and sugars in AML (Chattopadhyay *et al.*, 2001). The methanol extract of MPL showed the presence of flavonoids, tannins, steroids, triterpenoids and saponin (Chattopadhyay *et al.*, 2002a). Further fractionation and purification of the crude extracts from AML yielded three major (A, B and C) along with a minor fraction of some fatty acids; while the extract of MPL yielded two major (A and B) and a minor fractions of fatty acids, as reported earlier (Chattopadhyay *et al.*, 2001; 2002a). Further purification of the fractions yielded β-sitosterol (A), ursolic acid (B) and β-D-glucoside (C) from AML and ursolic acid (A) and β-sitosterol (B) from MPL. All the extracts as well as their fractions were then stored in a dessicator and dissolved in propylene glycol for this study.

Animals

Adult albino rats (Wistar strain) of both sexes, weighing 180 - 200 g each and Swiss albino mice of both sexes, weighing 20 - 25 g each were used. The

animals were kept in the animal house in the Department of Pharmacology, Dr. B. C. Roy P.G. Institute of Basic Medical Sciences, University College of Medicine, Calcutta University and maintained under standard water and diet *ad libitum*. All the animals were allowed at least one-week acclimatization period before the experiment.

Analgesic activity

Acetic acid-induced writhing test

This test was performed following the method of Koster and Anderson (1959). Swiss albino mice of either sex were divided into thirteen groups of ten animals each. The first two groups of animals received either propylene glycol (5 mg/kg) or aspirin (100 mg/kg) intraperitoneally. While the third to eighth group received either methanol extract of AML or MPL at doses of 100, 200 and 300 mg/kg, and the ninth to eleventh groups of animals received fraction A (β-sitosterol), B (ursolic acid) and C (β-D-glucoside) of AML at 50 mg/kg while the last two groups were administered with fraction A (ursolic acid) and B (β-sitosterol) of MPL at 50 mg/kg intraperitoneally, 60 min before i.p injection of 0.6% v/v acetic acid solution at a dose of 10 ml/kg. Immediately after the administration of acetic acid, the numbers of writhing or stretches (a syndrome, characterized by a wave of contraction of the abdominal musculature followed by extension of hind limbs) were counted for 15 min. A reduction in the writhing number compared to the control group was considered as evidence for the presence of analgesia, which was expressed as percent inhibition of writhing. Data's were calculated according to the formula:

$$A - B/A \times 100$$

Where A = Mean number of writhes produced by the control groups, and B = Mean number of writhes produced by the test groups.

Tail immersion test

Swiss albino mice of either sex were divided into thirteen groups of ten animals each. Propylene glycol at 5 mg/kg, methanol extracts of AML and or MPL at the doses of 100, 200 and 300 mg/kg, fraction A, B and C of AML or fraction A and B of MPL at 50 mg/kg, and the analgesic drug pethidine at 5 mg/kg were administered intraperitoneally. The tail (up to 5 cm) was then dipped into a pot of water maintained at $55 \pm 0.5^{\circ}C$. The time in seconds to withdraw the tail out of water was taken as the reaction time and the reading was taken after 30 min of administration of the test drugs. (Ghosh, 1984).

Tail flick test

Wistar albino rats of either sex weighing between 180 - 200 g were divided into thirteen groups of ten animals each. The tail of the rat was place on the nichrome wire of an analgesiometer (Techno, Lucknow, India) and the time taken by the animal to withdraw (flick) its tail from the hot wire was taken as the reaction time. The methanol extract of AML or MPL in doses of 100, 200 and 300 mg/kg, fraction A, B and C of AML or fraction A and B of MPL at 50 mg/kgand pethidine at 5 mg/kg were injected intraperitoneally. Propylene glycol at 5 ml/kg was served as vehicle control. Analgesic activity was measured after 30 min of administration of test and standard drugs (Ghosh, 1984).

Anti-inflammatory activity

The anti-inflammatory activity of methanol extracts of AML and MPL and their fractions was evaluated by carrageenan-induced paw oedema (Winter *et al.*, 1962; Arunachalam *et al.*, 2002) and dextran-induced paw oedema in rats, as acute and subacute model of inflammations respectively. Indomethacin (10 mg/kg *p.o.*) was used as standard drug for comparing anti-inflammatory effect in both acute and sub acute models of inflammation. Ten rats were used for these studies in each treatment group.

Statistical analysis

The results were analyzed for statistical significance

using the unpaired two-tailed student's *t*-test (Woodson, 1987).

RESULTS

Analgesic activity

Effect on acetic acid induced writhing test

The results of acetic acid induced writhing test with the methanol extract of AML and MPL in Swiss albino mice are presented in Table 1. The results indicate that the inhibition of writhing reflexes was 24.09% at 100 mg/kg and 49.63% at 200 mg/kg doses of methanol extract of AML; while the writhing reflexes inhibition was 0% in vehicle control group and 75.66% in aspirin treated group. The inhibition percentage was highest (74.69%) at 300 mg/kg dose, which is nearly equal and highly comparable to the inhibition given by the standard drug aspirin (75.66%). On the other hand, the writhing reflex inhibitions with AML fractions are 69.87% (A), 75.42% (B) and 68.19% (C) respectively. The results of the writhing reflex inhibition with methanol extract of MPL revealed that extract produced 21.2%, 51.12% and 73.97% inhibition at 100, 200 and 300 mg/kg doses respectively. While the writhing reflex inhibition with fractions at 50 mg/kg doses are 74.93% (A) and 70.60% (B) respectively, compared with the inhibition recorded by aspirin (75.66%). Here the significant inhibition of writhing reflexes for both the plants was found at 300 mg/kg dose of methanol extract and 50 mg/kg of fractions, and the data are highly significant in comparison to that of aspirin treated group (Table 1).

Effect on tail flick and tail immersion tests

The results of Tail flick tests, presented in Table 1 showed that the methanolic extract of AML had the reaction time of 2.75 sec at 100 mg/kg, 3.21 sec at 200 mg/kg and 3.98 sec at 300 mg/kg respectively; while the reaction time for vehicle control group was 2.25 sec and in pethidine control group was 4.20 sec. On the otherhand, reaction time at 50 mg/kg dose of fractions was 3.78 sec (A), 3.43 sec (B) and 3.41 sec (C) respectively. Here also the extract

Table 1. Analgesic activity of *Alstonia macrophylla* and *Mallotus peltatus* leaf extract and their fractions by physical and chemicals methods

Treatment	Dose (mg/kg)	Tail flick (Reaction time in sec)	Tail immersion (Reaction time in sec)	Number of writhing (% Inhibition)
Propylene glycol	5 ml/kg	2.25 ± 0.14	2.32 ± 0.16	41.50 ± 0.50
Aspirin	100	-	-	10.10 ± 0.43 (75.66)
Pethidine	5	4.20 ± 0.18	4.48 ± 0.12	-
	100	2.75 ± 0.04	2.64 ± 0.03	31.50 ± 0.34 (24.09)
Methanol extract of AML	200	3.21 ± 0.08	3.18 ± 0.02	20.90 ± 0.34 (49.63)
	300	3.98 ± 0.12	3.72 ± 0.18	10.50 ± 0.34 (74.69)
Fraction A	50	3.78 ± 0.14	3.54 ± 0.14	12.50 ± 0.04 (69.87)
Fraction B	50	3.43 ± 0.02	3.81 ± 0.04	10.20 ± 0.03 (75.42)
Fraction C	50	3.41 ± 0.04	3.61 ± 0.06	13.20 ± 0.22 (68.19)
	100	2.50 ± 0.13	2.48 ± 0.02	32.70 ± 0.36 (21.20)
Methanol extract of MPL	200	3.02 ± 0.06	3.05 ± 0.02	20.70 ± 0.43 (51.12)
	300	3.73 ± 0.10	3.65 ± 0.16	10.80 ± 0.34 (73.97)
Fraction A	50	3.69 ±0.21	3.65 ± 0.16	10.40 ± 0.02 (74.93)
Fraction B	50	3.01 ± 0.12	3.02 ± 0.12	12.20 ± 0.04 (70.60)

Values are mean ± SE, n = 10, $p < 0.001$ vs Control, Student's *t*-test. AML, *Alstonia macrophylla* leaf; MPL, *Mallotus peltatus* leaf.

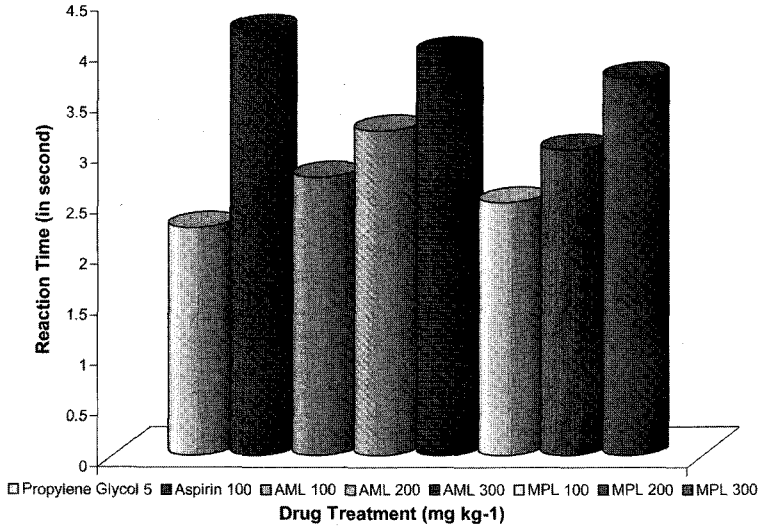

Fig. 1. Analgesic activity of *Alstonia macrophylla* and *Mallotus peltatus* leaf extracts by tail flick in Wistar rats.

at 300 mg/kg and fractions at 50 mg/kg doses significantly increased the reaction time compared to the pethidine (4.20 sec) group. The methanol extract of MPL revealed that the reaction time was 2.50 sec, 3.02 sec and 3.73 sec with 100, 200 and 300 mg/kg dose respectively; while the reaction time with fraction A was 3.69 sec and 3.01 sec with B of MPL at 50 mg/kg (Fig. 1).

The tail immersion test results revealed that the reaction time was 2.64 sec, 3.18 sec and 3.72 sec respectively with 100, 200 and 300 mg/kg of AML extract; while MPL extract at the same doses showed reaction time as 2.48 sec, 3.05 sec and 3.65 sec respectively, compared to the vehicle (2.32 sec) and the drug control (4.48 sec) group (Table 1). Here the reaction time for fraction A, B and C of AML was 3.54 sec, 3.81 sec and 3.61 sec respectively and reaction time for fraction A was 3.65 sec and B was 3.02 sec at 50 mg/kg dose (Fig. 2). All these data are highly significant with respect to the control groups.

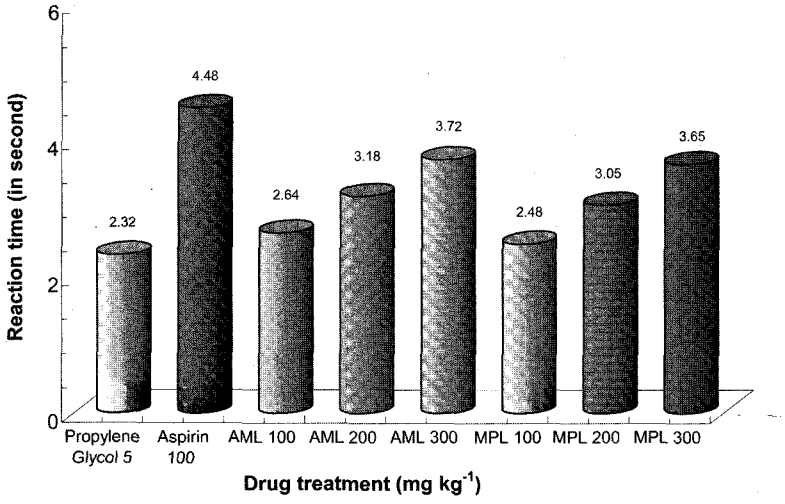

Fig. 2. Evaluation of analgesic activity of *Alstonia macrophylla* and *Mallotus peltatus* leaf extract in albino mice by tail immersion method

Table 2. Anti-inflammatory activity of methanol extract of *Alstonia macrophylla* and *Mallotus peltatus* leaf and their fractions in rats

Treatment	Dose (mg/kg)	Carrageenin-induced Rat paw oedema (% Inhibition)	Dextran-induced Rat paw oedema (% Inhibition)
Propylene glycol	5 ml/kg	5.26 ± 0.39	4.10 ± 0.17
Indomethacin	10	1.71 ± 0.31* (67.49)	1.60 ± 0.03* (67.77)
	200	2.43 ± 0.21* (53.80)	3.20 ± 0.53* (53.80)
AML extract	400	1.80 ± 0.29* (65.77)	2.10 ± 0.41* (65.77)
Fraction A	25	2.74 ± 0.18* (47.90)	2.52 ± 0.18* (38.53)
Fraction A	50	2.31 ± 0.12* (56.08)	2.03 ± 0.14* (50.48)
Fraction B	25	2.40 ± 0.22* (54.37)	2.32 ± 0.23* (43.41)
Fraction B	50	1.88 ± 0.28* (64.25)	2.05 ± 0.17* (50.00)
Fraction C	25	2.62 ± 0.10* (50.19)	2.23 ± 0.21* (45.60)
Fraction C	50	2.06 ± 0.14* (60.83)	1.93 ± 0.11* (52.92)
	200	2.38 ± 0.17* (54.75)	3.43 ± 0.33** (16.34)
MPL extract	400	1.86 ± 0.27* (64.63)	1.95 ± 0.31* (52.43)
Fraction A	25	1.82 ± 0.12* (65.39)	1.65 ± 0.10** (60.06)
Fraction A	50	1.61 ± 0.12* (70.25)	1.57 ± 0.20* (65.15)
Fraction B	25	1.83 ± 0.20* (64.39)	1.62 ± 0.27** (60.48)
Fraction B	50	1.67 ± 0.30* (69.13)	1.62 ± 0.12* (60.21)

Values are mean ± SE, n = 10, $^{**}p < 0.05$, $p < 0.001$ vs Control, Student's *t*-test. AML, methanol extract of *Alstonia macrophylla* leaf; MPL, methanol extract of *Mallotus peltatus* leaf.

Antiinflammatory activity

Effect of carrageenin and dextran induced paw oedema

The results of the antiinflammatory activity of methanol extracts of AML and MPL were presented in Table 2. The results revealed that the methanolic extract of AML at 400 mg/kg exhibited maximum inhibition (65.77%) of paw oedema and its fractions at 50 mg/kg showed 56.08% (fraction A), 64.25% (fraction B) and 60.83% (fraction C) inhibition respectively in carrageenan-induced rat paw oedema; while indomethacin showed 67.49% inhibition of oedema volume after 4h of drug treatment (Table 2). The results of the dextran-induced rat paw oedema test showed that the oedema suppression by AML at 400 mg/kg was 65.77%. While the fractions at 50 mg/kg showed 50.48% (fraction A), 50.00% (fraction B) and 52.92% (fraction C) inhibition of edema respectively, whereas indomethacin produced 67.77% inhibition. The results of the antiinflammatory activity of MPL extract showed that at 200 and 400 mg/kg doses the paw oedema inhibition was 54.75% and 64.63% in carrageenan induced (acute) model and 16.34% and 52.43% in dextran induced (subacute) model respectively; while the inhibition with 50 mg/kg of fractions A and B of MPL was 70.25% and 69.13% (in acute model), and 65.15% and 60.21% (in subacute model) respectively, compared to the drug control group (67.49% and 67.77%).

DISCUSSION

In acetic acid induced writhing models, the numbers of writhing movements were significantly less in the mice treated with the methanol extract of AML and MPL and their fractions, when compared to that of propylene glycol treated control. When the effect of the extracts and its fractions were compared with the standard analgesic agent aspirin, the

results suggest that the extracts might have peripheral analgesic effect. The analgesic effects produced by the Tail flick and Tail immersion tests were comparable to that of pethidine treated control, suggesting that it may have central analgesic effect. The study also revealed that the leaf extracts of AML and MPL at 200 and 400 mg/kg, p.o and its fractions at 50 mg/kg, p.o. exhibited significant anti-inflammatory activity in all the experimental models. The results of the anti-inflammatory activity of both the plant extracts and their lipophilic fractions indicated moderate to strong anti-inflammatory activity. However, the polar fractions containing two major alkaloid picrinine and picralstonine do not have recognisable anti-inflammatory activity. Both the extract and their fractions showed significant anti-inflammatory activity comparable to that of indomethacin against carrageenan induced acute pedal edema and dextran induced oedema. The carrageenan induced paw oedema is believed to be biphasic, of which the first phase is mediated by early release of histamine and 5HT followed by the release of kinin in later phase (Castro *et al.*, 1968). On the other hand, dextran mediated inflammation (oedema) was reduced probably as a result of antihistaminic effects of the extract and its fractions, as dextran is known to cause inflammation through both histamine and serotonin (Ghosh *et al.*, 1963). This may be due to the presence of ursolic acid (fraction B of AML and fraction A of MPL), as ursolic acid isolated from *Melaleuca leucadendron* is reported to inhibit concavalin A induced histamine release (Tsuruga *et al.*, 1991). Earlier work has also shown that the antiinflammatory activity of *Phillyrea latifolia* L is due to ursolic acid, which inhibits cyclooxygenase and 5-lipooxygenase enzymes of arachidonate cascade (Diaz *et al.*, 2000). Here both the crude extract and fractions of *A. macrophylla* and *M. peltatus* leaf showed significant anti-inflammatory activity in a dose dependent manner in all the animal models tested.

Earlier reports indicated that *Alstonia macrophylla* leaves contain several alkaloids like affinisine, picrinine and picralstonine (Banerji *et al.*, 1972), hydroxyvincamajine, alstonerine, alstophylline, macroalstonine, talcarpine, vinconine, cabucraline (Ratnayake *et al.*, 1987), strietaminolamine, dihydro-methylstrictamine (Rahman *et al.*, 1988), quebrachidine (Asolkar *et al.*, 1992), cathafoline, methoxyakuammicine, methoxy-methylburnamine, lagumidine and alstolagumine (Abe *et al.*, 1993a; Abe *et al.*, 1993b), and oxindole N_b-demethylalstophylline (Rahman *et al.*, 1987), macroxine (Rahman *et al.*, 1991). The oxindoles from bark includes alstonal, N_b-demethylalstophyllal, N_b-demethylalstophylline, alstonisine and talcarpine (Wong *et al.*, 1996); methoxyaffinisine, methoxycathafoline and alstonerinal (Kam *et al.*, 1996; Kam and Choo, 2000); the bisindoles alstomacrophylline and alstomacroline and the monomeric indole 20-*epi*-antirhine and villastonine (Keawpradub and Houghton, 1997). The biological activities of most of these alkaloids are not yet been reported. The *Mallotus peltatus*, on the otherhand, is not well studied, however, it is reported that *M. philippinesis* contain phloroglucinol (Lounasmaa *et al.*, 1975) with antifillarial (Singhal and Khan, 1997), purgative and anthelmintic (Singh and Tewari, 1967; Verma and Hishikar, 1984) activities; while *M. oppositifolium* have antimicrobial activity (Ogundipe *et al.*, 2000). The mallotojaponin, a terpenoid of *M. japonicus* have cytotoxic, antitumor (Fujita *et al.*, 1990a; Arisawa *et al.*, 1994) and antiherpetic (Fujita *et al.*, 1990b) activities, while rottlerin have protein kinase inhibiting (Gschwendt *et al.*, 1994) activity. However, the recent phytochemical study with *n*-butanol fraction of methanol extract of AML showed that β-sitosterol, ursolic acid and β-sitosterol glucoside as major compounds. On the otherhand, the bioactive *n*-butanol part of MPL extract yielded ursolic acid and β-sitosterol as major compound. The ursolic acid, [(3b)-3-Hydroxyurs-12-en-28-oic acid], a pentacyclic amphiphilic triterpene having planner hydroxylated polycyclic structure, is reported to be ubiquitous in plant kingdom. The medicinal plants containing ursolic acid in the

form of free acid or aglycones for triterpenoid saponins have been used since antiquity (Price *et al.*, 1987; Mahato *et al.*, 1988; Wang and Jiang, 1992) and its use in folk medicine are multiple. Contemporary research revealed that ursolic acid possesses diverse pharmacological actions like anticancerous (Kim 1997), antitumorigenic and antimetastatic (Young *et al.*, 1995; Kim *et al.*, 1999), antiulcer (Gupta *et al.*, 1981; Wrzeciono *et al.*, 1985), antiinflammatory (Harbone and Bakter, 1993; Chattopadhyay *et al.*, 2002a), antihistaminic (Tsuruga *et al.*, 1991), analgesic (Kosuge *et al.*, 1985; Liu, 1995), CNS depressant (Duke, 1992; Chattopadhyay *et al.*, 2003; Chattopadhyay *et al.*, 2004), antimicrobial (Kowalewski *et al.*, 1976; Zaletova *et al.*, 1987; Collins and Charles, 1989; Newali *et al.*, 1996), and antiviral (Kashiwada *et al.*, 1998). Recent studies suggest that ursolic acid can induce cell cycle arrest at G1 phase concomitantly with apoptotic cell death mediated by caspase-3 (Harmand *et al.*, 2003). Ursolic acid isolated from several medicinal plants is reported to inhibit human leukocyte elastage (Ying *et al.*, 1991; Safayhi *et al.*, 1997), 5-lipooxygenase and cyclooxygenase (Najid *et al.*, 1992; Ringbom *et al.*, 1998) and concavalin-A induced histamine release (Tsuruga *et al.*, 1991) as a potent antiinflammatory agent. Studies also showed that ursolic acid is a potent and highly selective inhibitor of cyclic AMP-dependent protein kinase (Wang and Polya, 1996) and cyclic AMP phosphodiestarase (Schussler *et al.*, 1991). Cyclic AMP dependent protein kinase is involved in the regulation of several cellular processes like metabolism, cell division, single gene expression and development (Edelman *et al.*, 1987; Cohen, 1989; Karin and Smeal, 1992).

The polar fractions of methanol extract of AML and MPL also yielded β-sitosterol as another major compound. Several studies revealed that the plants can synthesize a number of sterols, of which campesterol, sitosterol and stigmasterol (Grunwald, 1980; Burden *et al.*, 1989) are common. These sterols contain an extra alkyl group at C-24 position in the side chain and occur either free, or as esters and with β-D glucosides (Grunwald, 1980). In human, sitosterol (24α-ethylcholesterol) acts as a plasminogen activator (Hagiwara *et al.*, 1984; Hoffmann and Klöcking, 1988) and promotes the formation of essential polyunsaturated fatty acids from linoleic acid (Leiken and Brenner, 1989), which is required for prostaglandin and leukotriene synthesis, and is important for cell mediated immune functions (Kinsella *et al.*, 1990). A number of studies showed that sitosterol have several biological effects like inhibition of HT 29 human colon cancer cell growth (Awad *et al.*, 1996), epithelial cell proliferation (Janezik and Rao, 1992), chemically induced colon tumours (Raicht *et al.*, 1980; Deschner *et al.*, 1982), mammary lesions (Pezzuto, 1995) and act as an antimutagenic agent (Raj and Katz, 1984; Pezzuto, 1995). Sitosterol and its glucoside have a proliferating effect on *in vitro* T-cell production at remarkably low level, with a synergistic enhancement (Bouic *et al.*, 1996). An enhanced T-cell proliferative response was observed after 4 weeks of daily oral supplementation with 60 mg sitosterol and 0.6 mg of its glucoside in human volunteers with a normal diet (Bouic *et al.*, 1996); which strongly suggests that sitosterol and its glucoside have beneficial effect on immune system. Several studies revealed that sitosterol have anti-inflammatory (Gupta *et al.*, 1980; Salama *et al.*, 1987; Rios *et al.*, 1989; Gupta *et al.*, 1996), antiulcer (Adami *et al.*, 1962; Okuyama and Yamazaki, 1983; Romero and Lichtenberger, 1990; Xiao *et al.*, 1992), antidiabetic (Ghosal, 1985; Ivorra *et al.*, 1989; Marles and Farnsworth, 1995) and anticancer activity (Hartwell, 1976; Nozaki *et al.*, 1986; Yasukawa *et al.* 1991; Janezik and Rao, 1992; Pezzuto, 1995; Awad *et al.*, 1996) and facilitate to control rheumatoid arthritis (Pegel, 1980). Both β-sitosterol and its glucoside stimulate human peripheral blood leucocyte proliferation and significantly increase in the number of helper T cells, cytokines, interleukin 2, γ-interferon and NK cells activity and are thereby useful in the therapy of immune dysfunction (Bouic *et al.*, 1997). Sitosterol in combination with its glucoside has also been used in the treatment of

benign prostate hyperplasia (Berges *et al.*, 1995). Sitosterol and its glucosides are isolated from many ethnomedicinal plants like *Serenoa repens* (Schöpflin *et al.*, 1966), *Pygeum africanum* (Longo and Tira, 1981), pumpkin seed (Schilcher and Schneider, 1990), *Harpagophytum procumbens, Silybum marianum, Ginkgo biloba, Panax* species and *Eleutherococcus senticosus*. These phytosterols can enhance "adaptive" immunity by stimulating "innate" immune system and is termed as the 'adaptogen' (Brekhman and Dardymov, 1969; Farnsworth *et al.*, 1985; Wagner, 1995), which promote overall health without the side effect and rapid response of drugs (Brekhman and Dardymov, 1969; Farnsworth *et al.*, 1985; Wagner *et al.*, 1994).

The present investigation for the first time, confirms that there is potential analgesic and anti-inflammatory activity in the crude leaf extract of *Alstonia macrophylla* and *Mallotus peltatus* leaves, which appears to be due to the action of either ursolic acid (fraction B of *A. macrophylla* and fraction A of *M. peltatus*) alone or the combination of all the fractions. However, to know the exact mechanism of its analgesic and antiinflammatory activity further investigation with purified compound is required.

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