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Full Length Research Paper

Phytochemical and phytotherapeutic assessment of Mallotus peltatus (Geist.) Muell. Arg. var acuminatus, India

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We have evaluated the phytochemical and bioactive nature of the leaves of *Mallotus peltatus* (Geist.) Muell. Arg. var *acuminatus* and *Alstonia macrophylla* Wall ex A. DC, the two major ethno medicines of the Onge, Nicobarese and other tribes of Andaman and Nicobar Islands, on the Bay of Bengal, India. Three major compounds were isolated from the polar fractions of the methanol extracts of *A. macrophylla* leaf namely -sitosterol (fraction A), ursolic acid (fraction B) and -sitosterol -D-glucoside (fraction C), along with a minor fraction containing alkaloid and fatty acids. On the other hand polar fractions of methanol extract of *M. peltatus* leaf yielded two major compounds, ursolic acid (fraction A) and -sitosterol (fraction B), along with a minor fraction containing fatty acids. The earlier studies reveal that both the extracts have antibacterial, antiinflammatory, antipyretic and analgesic activities, but the present study showed that the activities are due to fractions A and B of both the plants. Moreover, the extracts of both the plants had significant dose dependent antiinflammatory and antioxidant activity at nontoxic concentrations. It can be thus concluded that the terpenoid and - sitosterol, along with other minor constituent of the bioactive parts had a dose dependent therapeutic efficacy, justifying their use. However, further study can help to elucidate their mechanism of action.

Key words: Ethnomedicine, *Alstonia macrophylla*, *Mallotus peltatus*, pharmacology and phytochemistry, antibacterial, antioxidant, antiinflammatory activity.

INTRODUCTION

The plant *Alstonia macrophylla* wall ex A. DC (Apocynaceae) is a panatropical shrub used by the Onge, Nicobarese and Shompen tribes of Andaman and Nicobar Is-lands mainly for stomachache (Dagar and Dagar, 1991), and skin diseases (Bhargava, 1983; Chakravarty and Va-sudeva Rao, 1988); while the *Mallotus peltatus* var *acu-minatus* (Euphorbiaceae) is a tropical rain forest bushy shrub used by the tribes and local people for stomach and intestinal problems (Dagar and Dagar,1991; Chakra-borty and Vasudeva Rao, 1988), anticholeretic, vulnerary effect and antitrematodic activity (Ambasta, 1992). Both these plants have been evaluated for their bioactivities, and their rationales for using as ethnomedicines of Andaman and Nicobar Islands on the Bay of Bengal. The earlier studies revealed that the alcoholic extract of both these plants have antibacterial (Chattopadhyay et al., 2001; 2002), antiinflammatory (Arunachalam et al., 2002; Chattopadhyay et al., 2002, 2005a, 2006), and antipyretic (Chattopadhyay et al., 2005) activities. Interestingly, fractions made out of crude extract revealed that it can stop the flagellar motility of bacteria (Chattopadhyay et al., 2001), as well as the for -ward motility of human spermatozoa (Chattopadhyay et al., 2005b, 2005c). On the basis of above results we have for the first time analysed the primary phytochemicals of those ethnomedicinal plants, and tested the efficacy of these fractions for antibacterial, anti-inflammatory and antioxidant activities.

The phytochemical analysis requires fresh plant tissues

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and the plant materials under investigation should be plunged into boiling alcohol within minutes of its collection. The isolation and purification of plant constituents is mainly carried out using one, other, or a combination of four chromatographic technique viz. paper chromatography (PC), thin layer chromatography (TLC), high performance liquid chromatography (HPLC) and gas liquid chromatography (GLC), depending largely on the nature of the substances present. However, there is considerable overlap in the use of those techniques and often a combination of PC and TLC, and GLC, followed by HPLC may be the best approach for separating a particular class of phytochemicals. For preparative work, TLC preferred, while for isolation or large-scale separation column chromatography is useful to yield purified components in gram amounts.

In plant tissues the steroids are widely distributed either in free or in combined state as esters or glycosides; and sterols such as cholesterol and sitosterol occur very often as inseparable mixture with the corresponding hydrocarbons or dihydro/dehydro derivatives. Phytosterols are structurally distinct from animal sterols, so that discovery for certain animal sterols in plant tissues are most intriguing, and among the phytosterols -sitosterol is most abundant (Harborne, 1973). Previous reports indi-cated that herbal remedies (one or more medicinal plants) predominantly contain -sitosterol along with - and -amy-rin as triterpenes (Hoopers and Chandler, 1984). It has been reported that -sitosterol is present in various medi-cinal plants like *Ficus bengalensis* (Subramanian et al., 1978), *Simarouba versicolor* (Ghosh et al., 1977), *Tithonia tagitiflora* Dersf (Pal et al., 1976), *Morus alba* (Mulberry) cal-lus tissue (Kulkarni et al., 1970), *Artocarpus chaplasha* (Ma-hato et al., 1966), *Teucrium polium* L. (Capasso et al., 1983)
Trichodesma africanum L. (Omar et al., 1983) etc. Another common bioactive phytochemicals are „terpe-
noid" (or isoprenoid), a large and diverse class of natural organic chemicals similar to terpenes (hydro-carbons resulting from the combination of several isoprene units),

derived from five-carbon isoprene CH_2=C (CH_3)-CH=CH_2 units assembled and modified, differ from one another in functional groups and in their basic carbon skeletons. The carbon skeleton of triterpenoids had six isoprene units,

derived from the acyclic C_{30} hydrocarbon squalene, with cyclic structures, most being alcohol, aldehyde or carboxylic acid. Triterpenoids are colourless, optically active crystal, often with high melting point, and is difficult to characterize due to lack of chemical reactivity. A widely used test is the Libermann-Burchard reaction (acetic an-hydride plus

concentrated H_2SO_4), which produces red-dish-violet color at the junction of the solvent layers showed with most triterpenes, steroids, saponins and car-diac glycosides (Harbone, 1973). Derivatives, as they are cyclic co- mounds with 4 or 5 ring systems, except squa-lene, and both classes is termed as phenanthrene deriva-tives. They are thus related to the steroids and this partly expresses itself in their similar chromatographic beha-viour (Stahl, 1969). Many triterpenoids are known in plants and new ones are regularly being discovered and characterized, but so far only a few are known to be of widespread distribution, such as the pentacyclic triterpenoids and -amyrin and the derived acids like ursolic acid and oleanolic acid (Harbone, 1973) . The first report of the isolation of ursolic acid was from wax -like coating of apple skin (Sando, 1923). Ursolic acid or malol, have also been isolated and characterized from the leaves of *Ichnocarpus frutescens* R. Br. (Khan et al., 1995), roots of *Urtica dioica* (Gansser et al., 1995) and leaves of *Mitragyna speciosa* (Said et al., 1991) etc. This paper mainly deals with the identification of major bioactive phytochemical groups, the structural elucidation by different spectral methods, and the bioactivities of the isolated group and compounds from the leaves of two well known ethnomedicines *A. macrophylla* Wall ex A. DC and *M. peltatus* (Geist.) Muell. Arg. var *acuminatus*, used by the Onge (one of the oldest tribes) of Indian subcontinent.

MATERIALS AND METHODS

Plant material

The leaves of *A. macrophylla* Wall ex A. DC and *M. peltatus* (Geist.) Muell. Arg. var *acuminatus* were collected from the rain forests of Middle and South Andamans, India, during April, June and October 1999. The voucher specimens were identified and authenticated by the Taxonomists of Botanical survey of India, Port Blair. The leaves were dried in shade, pulverized by a mechanical grinder and passed through 40-mesh sieve to get the fine powder.

Preparation of extracts

Coarsely powdered dry leaves of both the plants (1 Kg) were successively extracted in cold with 95% methanol as solvent for 72 h at room temperature (Chattopadhyay et al., 2001; 2002) . The whole extract was collected in a 5 liter conical flask, filtered, and the solvent was evaporated to dryness under reduced pressure in an Eyela Rotary Evaporator (Japan) at 40 - 45 C. The w/w yield of the prepared extract was 8.9 0.21% and 8.7 0.21% respectively.

Preliminary phytochemical group test

The preliminary phytochemical group test of leaf extracts from both the plants was performed by the standard methods (Chattopadhyay et al., 2005; Pollock and Stevens, 1965; Trease and Evans, 1996; Plummer, 1985).

Test for alkaloids: In separate tubes small quantities (2 - 5 gm) of the leaf extract of both plants were treated with few drops of dilute hydrochloric acid (HCl) and filtered. The filtrates were treated with Mayer"s reagent (mercuric chloride and potassium iodide), and the formation of Yellowish buff colored precipitate indicated positive test for alkaloids.

Small quantities of leaf extract of both plants were separately treated with few drops of dilute HCl and filtered, and the filtrates were treated with Dragendroff"s reagent (Sodium iodide, basic bismuth carbonate, glacial acetic acid and ethyl acetate). An orange brown precipitate indicated the presence of alkaloids.

When small quantities of leaf extract of both plants were separately treated with few drops of dilute HCL and filtered, and the filtrates were treated with Wagner"s reagent (Iodine and potassium iodide) a reddish brown precipitate developed, suggesting the pre-

sence of alkaloids.

Small quantities of leaf extract of both plants were separately treated with few drops of dilute HCl and filtered, and the filtrates were treated with Hager‟s reagent (aqueous solution of picric acid), a yellowish precipitate demonstrated the presence of alkaloids.

Test for amino acids: Small quantities of leaf extract of both plants were dissolved in a few ml of distilled water separately and treated with Ninhydrin (tri-ketohydrindene hydrate) at pH 4 to 8, and the absence of purple coloration suggested the absence of amino acids.

Test for flavonoids and their glycosides: Small quantities of leaf extract of both plants dissolved in ethanol separately and was hydrolyzed with 10% sulphuric acid (H_2SO_4) and cooled. Then, the mixture was extracted with diethyl ether and divided into three portions in three separate test tubes for each extract. 1ml of diluted sodium carbonate, 1ml of 0.1M sodium hydroxide and 1ml of diluted ammonia solution were added to the first, second and third test tubes of both extracts respectively. The development of yellow color in each test tube, demonstrated the presence of flavonoid.

Test for steroids and triterpenoids: Libermann-Burchard reaction: The extracts (10 mg) of both plants were separately dissolved in chloroform (1 ml); and then 1 ml of acetic anhydride was added to the mixture followed by 2 ml of concentrated H_2SO_4. A reddish violet ring at the junction of the two layers confirmed the presence of triterpenoids and steroids.

Salkowski test: When concentrated H_2SO_4 was added to chloroform solution (1 ml) of the extracts of both plant extracts (10 mg each), a reddish-blue color was produced in the chloroform layer and green fluorescence in acid layer, suggesting the presence of steroids.

Test for reducing sugar: Small quantities (2 - 5 mg) of extracts of both plants separately dissolved in minimum amount of distilled water and filtered. Equal volume of Benedict‟s reagent were mixed with the filtrate in a test tube separately and heated for few minutes, a brick red precipitate confirmed the presence of reducing sugars. Small quantities of extracts (2-5 mg) of both plants separately dissolved in minimum amount of distilled water and filtered, and to the filtrate equal volume Fehling”s solutions in a test tube separately heated for few minutes, leading to the development of brickred color indicated the presence of reducing sugars.

Test for gums: Small quantities of extracts of both plants separately dissolved in minimum amount of distilled water and filtered. The filtrate was treated with equal volume of concentrated H_2SO_4, and then treated with 15% alcoholic solution of -naphthol (Molish”s reagent). The formation of red-violet ring at the junction of sulphuric acid layer and extract indicated the positive test for gums (Molish”s test).

Test for tannins: Small quantities of extracts of both plants separately dissolved in minimum amount of distilled water and filtered, and the filtrate when treated with 10% aqueous potassium dichromate solution a yellowish brown precipitate demonstrated the presence of tannins.

When the above filtrate was allowed to react with 10% aqueous lead acetate solution, a yellow color precipitate formation indicated the positive test for tannins.

Again when the above filtrate of extract are allowed to react with 1 ml of 5% ferric chloride solution, formation of greenish black coloration demonstrated the presence of tannins.

Tests for saponins: Small quantities of extracts of both plants dissolved separately in minimum amount of distilled water and shaken in a graduated cylinder for 15 min. Formation of stable foam suggested the presence of saponins.

Chromatographic separation of bioactive compounds

For this a glass column fitted with stopcock was used, while the absorbent (absorbing material) was the Silica gel G (60 - 120 mesh size, SRL) and the solvents were Petroleum ether, Chloroform and Methanol (AR, SRL).

The concentrated extract of leaves of both the plants was separately partitioned between *n*-butanol and water. The aqueous portion was lyophilized to dryness (30 gm for *A. macrophylla* and 32 gm for *M peltatus*) while the *n*- butanol part was evaporated under reduced pressure. The residue (weighing 25 gm or 35 gm), was then purified on silicagel (60 - 120 mesh, SRL) by column chromatography, and eluted with petroleum ether (PE), PE: $CHCl_3$ mixture and $CHCl_3$: MeOH mixture (at different ratios) and MeOH. All the eluted fractions were monitored by thin layer chromato-graphy (TLC) using pre-coated aluminium plates (E. Merck, Germany). Three major fractions A (9 gm), B (5 gm) and C (6 gm) along with a mixture of minor compounds from *A. macrophylla*; while from *M. peltatus* two major fractions A (9 gm) and B (13 gm) along with a mixture of minor compounds were isolated. The isolated ma-jor fractions were then purified by repeated silica gel column chromatography and were eluted by PE: $CHCl_3$ (1:1) and $CHCl_3$: MeOH (95:5) mixture. Chemically the fractions from *A. macrophylla* were phytosterol (fraction A), triterpene (fraction B) and steroidal glucoside (fraction C); while the compounds from *M. peltatus* were triterpenoid (fraction A) and steroid (fraction B).

The extraction of alkaloids was carried out following standard procedure (Kam and Tan, 1990; Kam et al., 1999). In brief, the grounded powdered leaves were exhaustively extracted with 95% EtOH at ambient temperature. The EtOH extract was then concentrated under reduced pressure, partitioned into dilute HCl, alkaloids were then taken into chloroform to give a basic fraction containing a mixture of alkaloids. The alkaloids were isolated by repeated fractionation using CC and centrifugal TLC on SiO_2. The solvent system used for CC was $CHCl_3$–MeOH, while the solvent systems for centrifugal TLC were hexane–Et_2O and hexane–$CHCl_3$,. The yields (g kg^{-1}) of the alkaloids from the leaf extract were: 1 (0.022) and 2 (0.016).

Reagents and chemicals

The pBR322 plasmid DNA, 1,1-diphenyl-2-picryl-hydrazyl radical (DPPH), B-nicotinamide-adenine dinucleotide (reduced NADH) was obtained from Sigma Aldrich Co., St. Louis, USA and other reagents, chemical, media and drugs were obtained from the respective manufacturers.

Microbes

117 bacterial isolates were collected from the patients attending Calcutta School of Tropical Medicines and the Peerless Hospital, Kolkata, and grown in blood agar or MacConkey agar.

Animals

Wistar albino mice (18 - 20 g) and rats (150 - 180 g) of either sex were housed in groups in standard metal cages, prior to pharmacological studies with free access to standard diet and water *ad libitum* for two weeks and were fasted overnight before the test, supplied with water *ad libitum*.

Toxicity test

The *in vitro* cytotoxicity of the extracts or fractions was evaluated by attachment and growth inhibition assay (Lipipun et al., 2003) in vero

cells (1.5 X 10^5 cells). The serial twofold dilutions of the extract/ fraction/lead was added to confluent cell monolayers and incubated for 72 h. The 50% toxicity concentration (TC_{50}) causing visible morphological changes in 50% of cells with respect to cell control were determined (Lipipun et al., 2003). *In vivo* toxicity of the extract or fractions was assessed by LD_{50} determination in mice model. Briefly, the mice was divided into 8 groups (n = 10). The extract/fraction will be administered i.p. at 50 - 200 gm/kg of body weight. All animals were observed for physical sign of toxicity for 24h, and the LD_{50} was calculated by the method of Reed and Muench (1938).

Antibacterial and Antifungal Sensitivity testing

The antibacterial sensitivity of the extract or fraction was determined by disk diffusion assay in Mueller Hinton agar plates containing 10^6 cfu ml^{-1} of bacteria (CLSI, 2005) with controls and the sensitivity was recorded by measuring the clear zones of growth inhibition on agar surface around the discs. MIC was determined by agar dilution method using 2- fold dilution of the extract or fraction and the lowest concentration of the extract/fraction which did not show any visible growth after macroscopic evaluation was considered as MIC (Chat-topadhyay et al., 2001).

Effect of the extract or fraction on growth of bacteria was determined with standardized (1 ml) suspension of bacteria (2x10^6 cfu ml^{-1}) in Mueller Hinton broth containing the extract or fraction (0-2000 µg ml^{-1}) and incubated at 37°C for 18h with shaking at 200 rpm. The extract was added to the mid- logarithmic phase of growth and 1.0 ml aliquots were withdrawn at intervals for OD_{540} determination and colony count (Chattopadhyay et al., 1998).

Antiinflammatory activity

The antiinflammatory activity was determined by carrageenan induced paw oedema and cotton pellate-induce granuloma in rats. The extracts (200 mg kg^{-1}), fractions (50 mg kg^{-1}), vehicle (propylene glycol 5 ml kg^{-1}) and indomethacin (10 mg kg^{-1}) were administered orally, the paw volume was measured at 0h and 3h after the injection, and the percentage inhibition of paw oedema was calculated. For cotton-pellet granuloma test the sterile cotton pellets (10 mg) implanted rats received extracts, indomethacin and vehicle orally for 7 consecutive days, and on 8^{th} day the pellets were removed, made free from extraneous tissues, incubated and dried. The increase in dry weight of the pellets was taken as measure of granuloma formation (Arunachalam et al., 2002).

Antioxidant activity

The free radical scavenging activity of the extract was tested by „DPPH quenching" method (Russo et al., 2001) using 50 µl DPPH (2 mg ml^{-1}), extracts (10 - 100 µl), and fractions (10 - 50 µl -sito-sterol or 5 - 25 µl ursolic acid) and ascorbic acid (10 - 50 l as stan-dard) in 3 ml methanol. The percentage reduction in absor-bance was calculated from the initial and final absorbance of each solution while EC_{50} value was calculated from the calibration curve of extract concentration vs. percent reduction in absorbance (Russo et al., 2001).

Scavenger effect on superoxide anion

Superoxide anion was generated *in vitro* using 1 ml assay mixture [100 mM triethanolamine-diethanolamine buffer (pH 7.4), 3 mM NADH, 25 mM EDTA, 12.5 mM $MnCl_2$, 10 mM -mercaptoethanol] with two-fold concentrations of the extract or fraction (0 - 50 µg ml^{-1}) and superoxide dismutase (SOD; 80 mU ml^{-1}) as standard. After 20 min incubation at 25°C, the decrease in absorbance was measured at = 340 nm.

DNA cleavage

The DNA cleavage test was done by the method of Russo et al (2001) using 10 µl reaction mixture containing 33 µM bp of pBR322 DNA in 5 mM phosphate buffer (10 mM NaCl, pH 7.4), extract (1000 µg ml^{-1}) or fractions (500 µg ml^{-1}) and 1 mM DMSO (reference compound). Immediately prior to UV irradiation H_2O_2 (2.5mM) was added. The samples were irradiated for 5 min, mixed with 2 µl of dye mixture (0.25% bromophenol blue, 0.25% xylen cyanol FF and 30% glycerol in 40% sucrose solution), run with 1% agarose gel electrophoresis in Tris-acetate buffer (45 mM Tris-acetate, 1 mM EDTA), stained with ethidium bromide(1µg ml^{-1} for 30 min) and photographed on Polaroid microscope. Untreated plasmid DNA was used as control.

Statistical analysis

The results were expressed as mean, mean ± S.D. and SEM. The significance was evaluated by Student"s t-test compared with control (Chattopadhyay et al., 1988).

RESULTS AND DISCUSSION

The results of the preliminary phytochemical group tests of the leaves of *Alstonia macrophylla* and *Mallotus peltatus* have been furnished in Table 1.

The spectral analysis

UV absorption Spectrum of the steroidal compound: The Ultra Violet (UV) wavelength absorption spectrum of the substance in methanol solution, taken in a Hitachi Model No. 20 - 200 spectrophotometer, revealed no peaks above 210 nm, proving the absence of conjugated chromophoric system in *A. macrophylla* extract.

UV absorption Spectrum of the triterpenoid compound: The UV wavelength absorption spectrum of the substance in methanol solution was taken in a Hitachi Model No. 20- 200 spectrophotometer and No peaks could be detected above 210 nm, proving the absence of conjugated chromophoric system in both the plant extract.

UV absorption Spectrum of the steroid or steroidal glucosides: Ultraviolet wavelength absorption spectrum of the substance in methanol solution was taken in a Hitachi Model No. 20– 200 spectrophotometer. No peaks could be detected above 210 nm, proving the absence of conjugated chromophoric system in both the plants.

Infra Red (IR) spectra of isolated steroidal compound and its comparison with authentic sample: The IR Spectra of the isolated steroidal compound and authentic sample (-sitosterol) were taken with JASCO-FTIR spectrophotometer in potassium bromide discs, and the spectra were recorded in the region of 4000 cm^{-1} to 600 cm^{-1}. The spectral data of the isolated steroidal compound and -sitosterol are presented in Table 2. The IR spectrum of

Table 1. Preliminary phytochemical groups test of the leaves of *Alstonia macrophylla* and *Mallotus peltatus*.

Serial No	Phytoconstituents	*Alstonia macrophylla*	*Mallotus peltatus*.
1	Alkaloids	+	-
2	Steroids	+	+
3	Triterpenoids	+	+
4	Amino Acids	-	-
5	Flavonoids	+	+
6	Gums	-	-
7	Reducing Sugars	+	+
8	Tannins	+	+
9	Saponins	+	+

-, Absence; +, Presence.

Table 2. Infra Red (IR) Absorption spectra of the isolated steroidal compounds from *Alstonia macrophylla* with authentic sample of -sitosterol.

Isolated compound (V Max cm^{-1})	-sitosterol (V Max cm^{-1})
3417	3423
2937	2938
2868	2868
1463	1461
1378	1376
1051	1054
838	838
802	802

the compound shows absorption bands at 3417 and 1051 cm^{-1} indicating the presence of hydroxyl group. Other prominent peaks were at 2937, 2868, 1463 and 1378 cm^{-1} arising sitosterol -D-glucoside were taken with JASCO-FTIR spectrophotometer in potassium bromide discs.

The spectra were recorded in the region of 4000 cm^{-1} to 600 cm^{-1}, that shows the presence of absorption bands at 3394, 1024 and 1073 cm^{-1} indicating the presence of hy-droxyl group. Other prominent peaks were at 2935, 2870, 1460 and 1373 cm^{-1} arising from the hydrocarbon skele-ton. Since the IR spectrum of authentic sample -sitoste-rol -D-glucoside agreed very well with that of the isola-ted material, this proves the identity of the isolated mate-rial as -sitosterol -D-glucoside (Table 4).

IR spectra of the steroid compound and -sitosterol: The IR Spectra of the isolated steroidal compound from *M. peltatus* and an authentic sample (-sitosterol) were taken with JASCO-FTIR spectrophotometer in potassium from the hydrocarbon skeleton. The absorption bands at 838 and 802 cm^{-1} is due to the presence of C = C-H group.

Table 3. Infra Red (IR) Absorption spectra of the isolated triterpenoid compounds from *Alstonia macrophylla* with authentic sample of Ursolic Acid.

Isolated compound (V Max cm^{-1})	Ursolic Acid (V Max cm^{-1})
3427	3459
2931	2928
1688	1695
1025	1034
999	997

Table 4. Infra Red (IR) Absorption spectra of the isolated steroidal glycoside compounds from *Alstonia macrophylla* with authentic sample of -sitosterol glycoside.

Isolated compound (V Max cm^{-1})	-sitosterol glycoside (V Max cm^{-1})
3394	3395
2935	2934
2870	2870
1460	1461
1373	1373
1073	1072
1024	1024

IR Spectra of the triterpenoid compound and relevant authentic sample: The IR absorption spectra of the isolated triterpenoid were compared with the authentic sample of ursolic acid were taken with JASCO-FTIR Spectrophotometer in potassium bromide discs, and the IR spectrum of isolated compound agreed well with that of the authentic sample of ursolic acid. The spectrum indicates a typical IR spectrum of triterpenoid structure, as recorded in the region of 4000 cm^{-1} to 600 cm^{-1} and both showed close maximum absorption frequencies (V max) as shown in Tables 3 and 8. The absorption band at 3427 and 1688 cm^{-1} indicating the presence of hydroxyl and carboxyl groups. The band at 1025 and 999 cm^{-1} indicated the presence of -C-OH bond; while another peak at 2931 cm^{-1} arises from C-H bonds. For *M. peltatus* the IR spectrum shows the presence of absorption band at 3458 and 1696 cm^{-1} indicating the presence of hydroxyl and carboxyl groups. The band at 1033 and 996 cm- 1 indicated the presence of -C-OH bond. Another peak at 2929 cm^{-1} arises from the C-H bonds.

IR spectra of the steroidal glucoside compound and -sitosterol -D-glucoside: The IR Spectra of the isola-ted steroidal glucoside and an authentic sample of -bro-mide discs, and the spectra were recorded in the region of 4000 cm^{-1} to 600 cm^{-1}. The spectra of the iso-lated steroidal compound and -sitosterol have been shown in Figures 1 and 2 respectively and the spectral data are given in Table 9. The IR spectrum shows absorption bands

Table 5. ^{1}H-NMR spectra of the isolated steroidal compounds from *Alstonia macrophylla* and -sitosterol.

Position	^{1}H-NMR Isolated compound	^{1}H-NMR - sitosterol
3 (CH-OH)	3.53	3.53
6 (C=CH)	5.34	5.36
18,19,21,26,27,29(CH_3)	0.6 - 1.00	0.6 - 1.03
Other Protons	1.00 - 2.3	1.03 - 2.2

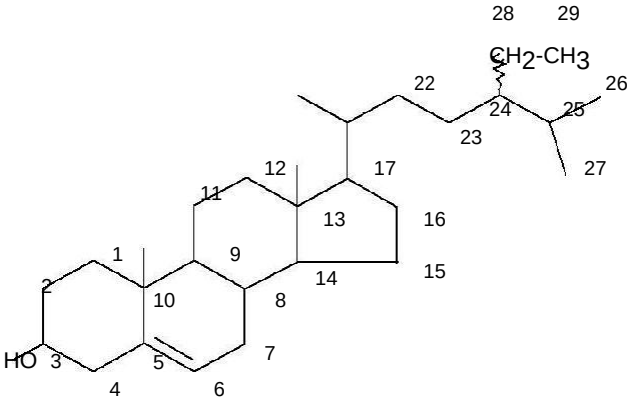

Figure 1. -Sitosterol.

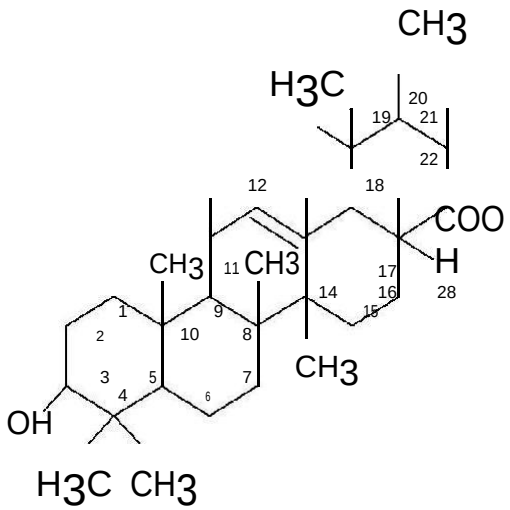

Figure 2. Ursolic Acid. [3 -hydroxy-urs-12-en-28-oic acid].

at 3426 and 1056 cm^{-1} indicating the presence of hydroxyl group. Other prominent peaks were at 2935, 2852, 1706 and 1462 cm^{-1} arising from the hydrocarbon skeleton; while the absorption bands at 965 and 802 cm^{-1} is due to the pre-sence of C = C-H group.

Nuclear Magnetic Resonance (NMR) spectra of the steroidal compound and authentic sample: The ^{1}H NMR spectra of the isolated compound and an authentic sample of -sitosterol were recorded in Bruker DPX-300 NMR spectrometer by taking the sample $CDCl_3$, and the spectra of the isolated compound and an authentic sample were identical. In the ^{1}H-NMR spectrum of the isolated compound in $CDCl_3$, shift appearing at 3.53 indi-cates the presence of CH –OH group at C-3 position of the structure. The olefinic proton at 6th position has peak at 5.34 and the six –CH_3 groups appeared between 0.6 - 1.00 region. The other protons appeared between 1.00 - 2.3. The shift positions of the isolated compound were almost identical with that of an authentic sample of -sitosterol (Table 5).

NMR Spectra of the isolated triterpenoid and ursolic acid: The ^{1}H NMR spectra of the isolated triterpenoid compound and an authentic sample of ursolic acid taken in Bruker DPX-300 NMR spectrometer in DMSO-d_6 (Dimethyl sulphoxide) solution, and the spectral data was summarized in Tables 6 and 10, while the spectra are shown in Figures 3 and 4 respectively. In the ^{1}H-NMR spectrum of the isolated compound in DMSO-d_6, the sig-nal appearing at 11.92 indicates the presence of a –COOH group at 28th position in the structure. The signal appearing at 3.3 agrees with the presence of - CH-OH at 3rd position. The – OH group accounting for the peak at 4.29. The presence of signal at 5.12 signifies the presence of a trisubstituted double bond (unsaturation). The seven –CH_3 groups appeared between 0.6 and 1.3. The shift positions of the isolated compound were almost identical with those of the authentic sample of ursolic acid for both the plants.

NMR spectra of the steroidal glucoside and authentic sample of -sitosterol -D-glucoside: The NMR spec-tra of isolated compound and authentic sample of -sito-sterol -D-glucoside were taken in Bruker DPX-300 NMR spectrometer by dissolving the samples in DMSO d_6 and found that the spectra were nearly identical. In the ^{1}H-NMR spectrum of the isolated compound in DMSO-d_6, the signal appearing at 5.33 indicates the presence olefinic proton at 6th position in the structure. These at 3.34-3.75 can be attributed to protons at 2"- 6" positions in sugar moiety. The doublet at 4.22 agrees with that expected for aromatic in sugar moiety. The six signals for the –CH_3 groups appeared in the spectra between 0.6 to 1.00. Other proton signals were located between 1.13 – 2.4 and 2.8 – 3.1 regions. The shift positions of the isolated compound were almost identical with those of the authentic sample of -sitosterol -D-glucoside (Table 7) except for a peak at 4.06, which appears to be less impurity.

NMR spectra of the steroid and authentic sample: The ^{1}H NMR spectra of the isolated compound and an authentic sample of -sitosterol were recorded in Bruker DPX-300 NMR spectrometer by taking the sample $CDCl_3$. The NMR spectra of the isolated compound and the authentic sample were identical. Here the ^{1}H-NMR spectrum of the isolated compound shows the shift at 3.53, that indicates the presence of CH –OH group at C-3 posi-

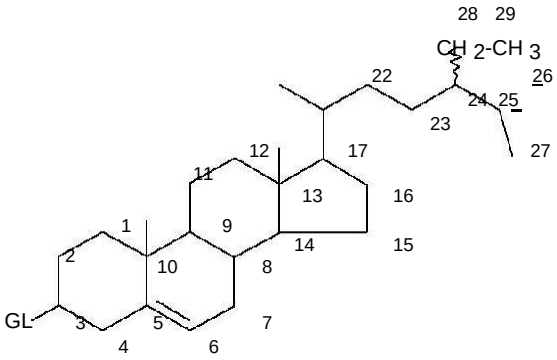

Figure 3. -Sitosterol -D-Glucoside.

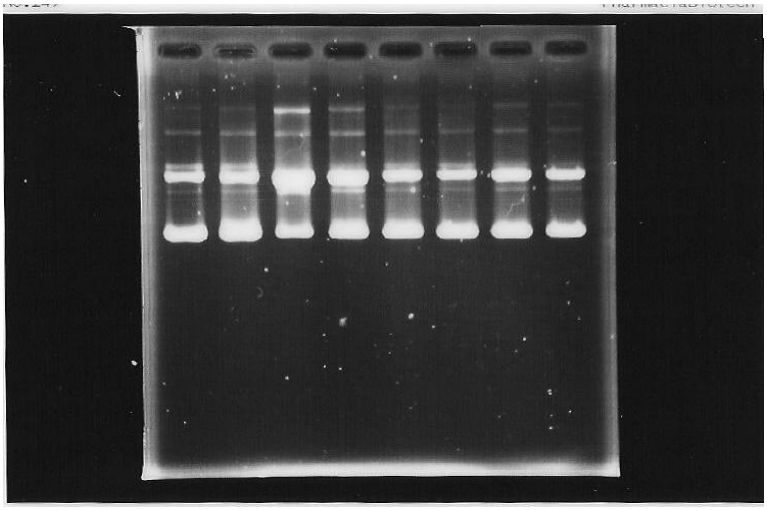

Figure 4. Effect of *A. macrophylla* and *M. peltatus* leaf extracts and their fractions at 500 and 1000 g ml^{-1} concentration on the protection of supercoiled DNA against OH generated by the photolysis of H_2O_2 (2.5 mM). Dimethylsulfoxide (1mM) was used as reference compound.
Lane 1: untreated DNA (Control); Lane 2: UV treated DNA (Control); Lane 3: DNA treated with 2.5 mM H_2O_2; Lane 4: DNA + H_2O_2 + DMSO; Lane 5: DNA + H_2O_2 + AML extract; Lane 6: DNA + H_2O_2 + MPL extract; Lane 7: DNA + H_2O_2 + -sito-sterol fraction; Lane 8: DNA + H_2O_2 + Ursolic acid fraction

Table 6. 1H- NMR spectra of the isolated triterpenoid com-pounds from *Alstoni macrophylla* and Ursolic Acid.

Position	^{1}H-NMR Isolated compound	^{1}H-NMR Ursolic Acid
3 (CH-OH)	3.33	3.33
28	11.92	11.92
12 (C=CH)	5.12	5.12
3 (CH-OH)	4.29	4.29
23,24,25,26,27,29, 30	0.6 - 1.3	0.6 - 1.3

tion. The olefinic proton at 6th position with a peak at 1.03 regions, while the other protons are between 1.00 - 2.3. The

Table 7. 1H-NMR spectra of the isolated steroidal glycoside compounds from Alstonia macrophylla with authentic sample of -sitosterol glycoside.

Position	^{1}H-NMR Isolated compound	^{1}H-NMR -sitosterol glycoside
6(C=C)	5.33	5.33
2" - 6" (Sugar moiety)	3.34 - 3.7	3.34 - 3.7
1" (Sugar moiety)	4.22	4.22
18,19,21,26,27,29	0.6 - 1.00	0.6 - 1.00
Other Protons	1.3 – 2.4	1.3 – 2.4

shift positions of the isolated compound were almost identical with that of an authentic sample of -sitosterol

Table 8. Infra Red (IR) Absorption spectra of the isolated triterpenoid compounds from *Mallotus peltatus* with authentic sample of Ursolic Acid.

Isolated compound (V Max cm^{-1})	Ursolic Acid (V Max cm^{-1})
3458	3459
2929	2928
1696	1695
1033	1034
996	997

Table 9. Infra Red (IR) Absorption spectra of the isolated steroidal compounds from *Alstonia macrophylla* with authentic sample of -sitosterol.

Isolated compound (V Max cm^{-1})	-sitosterol glycoside (V Max cm^{-1})
3429	34235
2935	2938
2852	2868
1706	1662
1462	1461
1056	1054
965	838
802	802

(Table 11).

Mass spectrum of the isolated steroidal compound: Mass spectrum (EI.MS) of the isolated steroid from leaves of *A. macrophylla* and *M. peltatus* were taken in a JEOL JMS600 Mass spectrometer separately. The compound showed the principal peak at *m*/*z* 414 (M+) with significant fragment ion peaks at *m*/*z* 396 (M-18), 381(M-33), 273 (M-side chain), 255 (M-side chain-18), 231 (M-side chain-42), 213 (231-H_2O). The intense peak with the highest mass number at *m*/*z* 414 is due to the parent molecular ion for -sitosterol. A less intense peak at *m*/*z* 400 signifies the presence of its lower homologue (Campesterol) in small amount.

Mass spectrum of the isolated triterpenoid compound: Mass spectrum (FAB MS) of the isolated triterpenoid compound from the leaves of *A. macrophylla* and *M. peltatus* were taken in a JEOL JMS600 Mass spectrometer separately. The isolated material showed significant peaks at *m*/*z* 439, 411, 393,248, 203 and 189 with 119 as the base peak. The molecular ion (protonated) peak was not significant in intensity though a somewhat prominent peak at *m*/*z* 479 may be assigned to a $[M^{+}Na]^{+}$ ion. However, the prominent peak at *m*/*z* 248 along with other peaks at *m*/*z* 207, 203 and 189 indicated the com-pound to be ursolic acid (or isomer).

Mixed melting point of the steroidal compound with authentic sample: The isolated steroidal compound melted at 136 - 137°C. When small amount of the isola-ted compound was mixed with an equal amount of au- thentic sample of -sitosterol, the mixture melted at 136 - 137°C without any depression.

Mixed melting point of the triterpenoid compound with authentic sample: The isolated triterpenoid compound melted at 285 - 287°C. Small amount of the isolated compound was mixed with an equal amount of authentic sample of ursolic acid and the mixture was allowed to melt, it shows that the mixture was melted at 285 - 287°C without showing any depression.

Mixed melting point of the steroidal glucoside compound with authentic sample: The isolated steroidal glycoside from *A. macrophylla* melted at 283 - 286°C. When small amount of the isolated compound was mixed with an equal amount of authentic sample of -sitosterol glucoside, the mixture melted at 283 - 286°C without showing any depression.

Mixed melting point of the steroid with authentic sample: The isolated steroidal compound from *M. peltatus* melted at 136 - 137°C, but when small amount of the isolated compound was mixed with an equal amount of authentic sample of -sitosterol and the mixture was allowed to melt, it melted at 136 - 137°C without showing any depression.

The crystalline material isolated from the leaves of *A. macrophylla* as fraction A and from *Mallotus peltatus* leaves as fraction B demonstrated positive test for steroid. All the recorded data such as UV, IR, ^{1}HNMR 9.5 spectrum and mixed melting point conclusively prove the identity of the isolated steroidal compound as " -Sitosterol" (Figure 1).

All the experimental evidence as furnished in the previous section (UV, IR, ^{1}H-NMR, Mass spectrum and mixed melting point with an authentic sample of Ursolic acid) suggests that the compound from fraction B of *A. macrophylla and* Fraction A of *M. peltatus* isolated is "Ursolic Acid" (Figure 2) test for a steroidal compound. All the present data such as IR and ^{1}HNMR and mixed melt-ing point conclusively prove the identity of the isolated compound as " -sitosterol -D-glucoside" (Figure 3).

The minor fraction we obtained contain alkaloid in minute amount and this fraction do not reveal any significant antibacterial, antiinflammatory and antioxidant activity studied, and thus, we didn"t proceed further for the isolations and purification of alkaloid.

Phytotherapeutic study

Toxicity study: The results of *in vitro* toxicity study showed that after 72h of treatment, the extracts at 3000 g ml^{-1} did not inhibit the vero cell growth or alters cell morphology (no rounding up, detachment or shrinking) compared to the drug free control. However, the cell toxic concentration (CTC_{50} ml^{-1}) was 6000 g ml^{-1} and the sub-

Table 12. Minimum inhibitory concentration spectra of *M. peltatus* and *A. macrophylla* leaf extracts.

Bacteria	No.	MPL extracts (g/ml)			AML extract(µg/ml)				MIC of Amoxycillin (g/ml)					
		128	256	512	64	128	256	512	0.25	0.5	8.0	64	128	256
Escherichia coli	15	-	-	12	05	01	02	02	-	02	01	01	05	03
Kleb pneumoniae	07	-	-	-	-	-	-	-	-	-	-	-	05	01
Salmonella typhi V*1*	07	-	-	-	-	-	-	-	-	-	-	-	01	01
Shigella dysenteriae	07	-	-	01	-	-	-	-	-	-	-	01	01	01
Vibrio cholerae	05	-	-	-	-	-	-	-	-	-	-	-	-	01
Proteus mirabilis	07	-	-	05	07	01	-	-	-	-	-	-	-	01
Proteus vulgaris	07	-	-	02	05	-	01	02	-	-	-	-	01	01
Pseudo aeruginosa	07	-	-	-	-	-	-	-	-	-	-	-	-	-
Staph aureus	15	10	04	01	-	-	-	02	-	05	01	03	03	01
Staph saprophyticus	05	02	01	02	-	-	-	-	01	01	-	02	-	-
Strepto faecalis	06	03	02	-	-	-	-	01	01	01	01	01	01	01
Bacillus subtilis	05	01	02	02	-	02	01	01	-	01	-	01	01	-
S. aureus 25923	01			+				+	+					
E. coli ATCC 25922	01		+				+				+			
P. aeruginosa 10662								+						+
TOTAL	95	16	09	25	17	0 4	04	08	02	10	03	09	18	11

toxic concentration was between 2000 - 3000 g ml^{-1}. The *in vivo* toxicity study revealed that the extracts are non-toxic up to 3.0 - 3.2 mg kg^{-1} body weight in albino Wister mice and rats.

Antibacterial activity: The sensitivity spectra of *M. peltatus* and *A. macrophylla* leaf extract, presented in Table 12, revealed that out of 95 isolates tested MPL extract inhibited 50 isolates of at 128 - 512 µg/ml and 33 isolates are sensitive to AML extract at 64 - 512 µg/ml concentrations respectively, while rest are either sensitive to 1000 µg/ml or resistant. Interestingly, 53 isolates are sensitive to Amoxycillin with an MIC of 0.25 - 256 g ml^{-1} (Table 12). Significantly, 5 *E. coli* and 6 *Proteus mirabilis* isolates are sensitive to AML extract at 64 g ml^{-1} but resistant to amoxycillin, even up to 1000 µg ml^{-1}. Further study showed that the growth of 3 susceptible bacterial isolates were decreased by increasing concentration, and were completely inhibited at their MIC values. Thus both the extracts exhibited bacteriostatic activity at lower concen-tration but cidal at higher concentration and the MBC was 2 folds higher than MIC.

The results are mean values of triplicate tests repeated three times after 24-72 h of incubation at 37^{o}C. Diameter of inhibition zones varies from 10 14 mm S.D. at MIC concentrations. MPL, *M. peltatus* leaf; AML, *A. macrophylla* leaf.

Antiinflammatory activity: The results presented in Table 13 showed that both AML and MPL extracts had significant antiinflammatory activity at 400 mg ml^{-1} in carrageenan-induced paw oedema model, with a maximum inhibition of 65.77% and 64.63% respectively. Interestingly, the inhibition by fraction B (64.25% and 69.13%) is nearly similar to indomethacin (67.77%) . In cotton pellet granuloma tests AML and MPL extract at400 mg kg^{-1} inhibited granuloma weight by 52.96% and 1.54%, while fraction A of MPL gave highly significant inhibition of granuloma weight (60.25%) compared to indomethacin (54.07%).

Antioxidant activity: The methanol extracts of both AML and MPL are found to bleach stable DPPH radical having an odd electron (give a strong absorption band at 517 nm; deep violet) that becomes paired off in presence of extract or fraction (free radical scavenger), the absorption vanishes and the resulting decolorization is stoichiometric with respect to the number of electrons taken up. The results revealed that the quenching more pronounced with the fractions and 5 g ml^{-1} ursolic acid corresponds to the action of 80 mU ml^{-1} of SOD (data not shown). The effect of AML and MPL extracts (1000 µg ml^{-1}) and their fract-ions (500 µg ml^{-1}) on pBR322 DNA cleavage, presented in (Figure 4), showed that the plasmid DNA had two bands (lane 1), the faster moving band corresponded to the native form of supercoiled circular DNA (scDNA) and the slower moving capacity of AML extracts (200 g ml^{-1}) and ursolic acid fraction (100 g ml^{-1}) was equivalent to ascorbic acid (25 M). The results of the superoxide sca-venging effect was band of open circular DNA (ocDNA). The UV irradiation of DNA in presence of H_2O_2 (lane 3) resulted in the cleavage of scDNA to ocDNA and linear DNA (lin DNA), indicating that OH generated from UV photolysis of H_2O_2 produced DNA strand scission. The addition of AML (lane 5), MPL (lane 6), -sitosterol (lane 7) and ursolic acid (lane 8) suppressed the formation of lin DNA and induced a partial recovery of scDNA. The ac-

Table 13. Antiinflammatory activity of AML and MPL extract on paw oedema and Granuloma formation in rats.

Treatment	Dose (p.o)	Paw Volume		% Inhibition	
		AML	MPL	AML	MPL
Carrageenan-induced paw oedema					
Propylene glycol	5ml kg^{-1}	5.26 ± 0.39	5.26 ± 0.39	-	-
Indomethacin	10 mg kg^{-1}	1.71 ± 0.31	1.71 ± 0.31	67.77	67.77
Methanol extract	400 mg kg^{-1}	1.80 ± 0.29*	1.86 ± 0.27*	65.77	64.63
Fraction A	50 mg kg^{-1}	2.31 ± 0.12*	1.61 ± 0.12*	56.08	70.25
Fraction B	50 mg kg^{-1}	1.88 ± 0.28*	1.67 ± 0.30*	64.25	69.13
Cotton-pellate induced Granuloma test					
Treatment	**Dose (p.o)**	**Weight of granulation**		**% Inhibition**	
		AML	MPL	AML	MPL
Propylene glycol	5 ml kg^{-1}	70.42 ± 3.60	70.42 ± 3.60	-	-
Indomethacin	10 mg kg^{-1}	32.34 ± 1.71*	32.34 ± 1.71*	54.07	54.07
Methanol extract	400 mg kg^{-1}	33.12 ± 1.52*	34.12 ± 1.26*	52.96	51.54
Fraction A	50 mg kg^{-1}	34.50 ± 0.64***	30.55 ± 0.12*	51.00	60.25
Fraction B	50 mg kg^{-1}	34.75 ± 0.81***	31.97 ± 0.20*	50.65	55.11

Values are mean ± SE, n=6, *P<0.05, **p<0.01, ***P<0.001 compared with control, Student"s t-test. AML, *Alstonia macrophylla* leaf; MPL, *Mallotus peltatus* leaf.

tion of extracts was comparable to 1 mM did not induce DNA damage (lane 2). DMSO (lane 4) and UV irradiation without H_2O_2 for 5 min

DISCUSSION

Decoction of AML and MPL were used in folk medicine of Onge tribes of Little Andaman Island to cure diverse ailments including infections, and in most infections localized inflammation occur; hence, we have tested the extracts for antibacterial and anti-inflammatory activity. The antioxidant activity was tested as the decoction and poultice are used to prevent gastrointestinal ailments and skin irritation. The phytochemical analysis of *A. macrophy-lla* leaf extract yielded flavones, triterpenoids, tannins, sterols, saponin and alkaloid; while *Mallotus peltatus* leaf extract had tannins, sterols, flavones and triterpenoids. The TLC, column chromatographic, spectroscopic and crystallographic study revealed that the polar fractions of *A. macrophylla* leaf contain picrinine and picralstonine (in very minute amount), while the lipophilic fractions had -sitosterol (fraction A), ursolic acid (fraction B) and -sitosterol glucoside (fraction C) along with some fatty acids. The bioactive lipophilic part of *M. peltatus* leaf extract contains ursolic acid (fraction A) and -sitosterol with some fatty acids (fraction B).

The previous investigation revealed a moderate degree of antibacterial activity of AML and MPL extracts and the present study confirm that the antibacterial activity is possibly due to the action of fraction A or B alone or in combination, as evident by the MIC and growth inhibition curve. Carrageenan-induced paw oedema, used for evaluating antiinflammatory potential of plant extracts is biphasic, where the initial phase is mediated by the early release of histamine and serotonin followed by kinin and bradykinin, and finally by prostaglandin and lysosome (Arunachalam et al., 2002), therefore, the anti-inflammatory activity of these extracts is due to its antihistamine or antiserotonin nature. The cotton-pellet granuloma test reflects the efficacy of both the extracts to inhibit the proiterative phase (increase in number of fibroblasts and synthesis of collagen and mucopolysaccharides during granuloma tissue formation) of inflammation process. Our data on free radical scavenging study with AML and MPL extracts or fractions containing flavonoids, sterol and tan-nins exhibits interesting dose dependent antioxidant pro-perties as evident from its capacity to scavenge DPPH and superoxide anion (SOD) and to protect plasmid DNA from hydroxyl radicals (OH) induced cleavage. These re-sults may explain, in part, its use in traditional medicine for gastrointestinal ailments.

Reports indicate that different parts of *A. macrophylla* contain various alkaloids like picrinine, picralstonine, 0-benzoyl vincamajine and quebrachidine (Chattopadhyay et al., 2001; Arunachalam et al., 2002), while *Mallotus* species contain -sitosterol, moretenol, rottlerin and mallotucins (Chattopadhyay et al., 2005) . However, the present study for the first time confirmed that the bioactive *n*-butanol part of methanol extract of AML contains -sitosterol, ursolic acid and -sitosterol glucoside, while MPL extract had ursolic acid, -sitosterol and some fatty acids as major compounds. Saponins are aglycone linked to one or more sugar chains, and have two groups, one contains a steroidal aglycone, and the other a triterpenoid aglycone (Price et al., 1987) biosynthesized from a common precursor squalene. Triterpenoids, like steroids, have many biological effects. Ursolic acid [3 - hydroxy-urs-12-en-28-oic acid], a pentacyclic amphiphilic triterpenoid with planner hydroxylated polycyclic structure, is ubiquitous in the me-dicinal plants (Price et al., 1987; Mahato et al., 1988; Wang and Jiang, 1992) used since antiquity. Ursolic acid, the aglycones for many triterpenoid saponins have been shown to be responsible for various bioactivity and the traditional uses of plants containing u

solic acid in folk medicines are multiple. Contemporary research revealed that ursolic acid isolated from medicinal plants possesses diverse pharmacological actions like anticancerous (Kim, 1997), metastatic and anti-tumorigenic (Young et al., 1995), antiulcer (Gupta et al., 1981; Snyckers and Fourie, 1984; Wrzeciono et al., 1985), hypoglycaemic (Ivorra et al., 1989), antihyperlipidemic (Ma, 1986), antimicrobial (Zaletova et al., 1986; Collins and Charles, 1989; Newali et al., 1996), antiviral (Kashiwada et al., 1998), anti-inflammatory (Harbone and Bakter, 1993), antihistaminic (Tsuruga et al., 1991), analgesic (Kosuge et al., 1985), CNS depressant (Duke, 1992), hepatoprotective (Shukla et al., 1992), cardiotonic sedative and tonic effects (Liu, 1995). The acidic triterpene ursolic acid inhibits mutagenicity (Niikawa et al., 1993), lipooxigenase and cyclooxygenase *in vivo* (Najid et al., 1992; Ringbom et al., 1998), leucocyte elastage (Ying et al., 1991), histamine release from mast cells (Tsuruga et al., 1991) and leukaemic cell proliferation (Si-mon et al., 1992). It can also protect from Cd^{2+}-induced liver damage (Ferriola et al., 1989) by increasing hepatic metallothionein level (Liu et al., 1994). Ursolic acid is also reported to be a potent and highly selective inhibitor of cyclic AMP-dependent protein kinase (Wang and Polya, 1996), and 3", 5"-cyclic adenosine monophosphate phosphordiestarase (Schussler et al., 1991) . Cyclic AMP depen-dent protein kinase is involved in the regulation of cellular processes like metabolism, cell division, single gene expression and development (Edefman et al., 1987). Japanese researchers recently reported that ursolic acid se-lectively inhibits mammalian DNA polymerases and , as well as human DNA topoisomerases I and II (Mizushina, 2000). The antiangiogenic (Sohn et al., 1993) antioxi-dant (Balanehru and Nagarajan, 1992) and antioxida-tive (Kim et al., 1996) properties as well as induction and selective inhibition of apoptosis (Kim Dong-Kyoo et al., 2000; Choi *et al*., 2000) by ursolic acid have also been reported. Another major compound isolated from polar fractions of the methanol extract of *A. macrophy-lla* and *M. peltatus* leaves is -sitosterol. This phytoste-rol and its glucoside have anti-inflammatory, antipyre-tic, antiulcer, antidiabetic and anticancerous activities and facilitate to control rheumatoid arthritis (Pegel, 1980; Gupta et al., 1996).

Recent reports indicate that -sitosterol and its gluco-side stimulate human peripheral blood leucocyte prolife - ration and cause significantly increase in helper T cells:
Th1 and Th2, cytokines, interleukin 2, - interferon, and NK cells activity; and can lyse cancer cells and are thereby useful in the therapy of several diseases caused by immune dysfunction (Bouic et al., 1997).

The earlier studies with crude extracts of both these plants showed its antibacterial (Chattopadhyay et al., 21001; 2002; 2005), antiinflammatory (Arunachalam et al., 2002; Chattopadhyay et al., 2002), antipyretic (Cha - ttopadhyay et al., 2005a) and antimotility activities Chattopadhyay et al., 2005b,c) are perhaps due to the combined activities of these phytoconstituents. However, the present study for the first time confirms that the *A. macrophylla* and *M. peltatus* leaf extracts have antibacterial, antiinflammatory and antioxidative activity at different dose level, probably due to their fractions containing ursolic acid and sitosterol alone or in combination. However, to understand the mechanism of diverse activity of these extracts or fractions further investigation is required. In conclusion, the vernacular medicinal use of these plants to treat several ailments gives us a multiple advantage in primary health care.

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