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Original Article

Evaluation of analgesic and anti-inflammatory activity of *Bridelia retusa* (Spreng) bark

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ABSTRACT

Several species of *Bridelia* have been used in the condition of pain & arthritis in Indian folk medicine. Present study revealed the preliminary phytochemical investigation and evaluation of analgesic, anti-inflammatory and anti-arthritic activity as well as underlying mechanism of bark of *Bridelia retusa* Spreng. (Euphorbiaceae). The bark was subjected to extraction using pet.ether, ethyl acetate and acetone. All the extracts were significantly inhibit abdominal writhings response and licking time in late phase of formalin test. Extracts could also significantly inhibit mean paw edema of rats induced by carrageenan & histamine at dose of 200 & 400 mg/kg, i.p. Test materials also showed significant dose dependent reduction in cotton pellet granuloma & acetic acid induced vascular permeability at 400 mg/kg. Oral administration of *B. retusa* fractions in CFA induced arthritic rats, physical, biochemical and hematological parameters observed in arthritic animals were altered significantly to near normal condition. The maximum paw edema inhibition at day 21 was observed at 400 mg/kg. It also proved significant protection against protein denaturation & RBC membrane damage.

The GC-MS analysis of EA extract revealed the presence of β-sitosterol, stigmasterol, lupeol and friedelin (Pentacyclic triterpenoid). Therefore present study has demonstrated the analgesic; anti-inflammatory and anti-arthritic activities of *B. retusa* bark and suggested that the molecular membrane might be associated with inhibition of biochemical and hematological parameters. Overall bioactive profile of *B. retusa* used phytomedicine in future for inflammatory conditions.

1. Introduction

Inflammation and pain are common nonspecific manifestations of many diseases. It is a defence mechanism aimed to remove the injurious stimuli and initiate the tissue healing process.[1] Various endogenous mediators such as histamine, serotonin, bradykinin, prostaglandins, etc. are most abundant in inflammatory cells and among them prostaglandins are ubiquitous substances that indicate and modulate cell and tissue responses involved in inflammation.[2] However, prolonged inflammation can lead to numerous diseases including rheumatoid arthritis (RA), psoriasis, and inflammatory bowel disease. RA, a chronic inflammatory disease which is characterized by immune-mediated inflammatory sinusitis involving cartilage and bone destruction, joint malformation, functional impairment results into continued swelling around the joint, pain, synovial hyperplasia, pannus formation, and morphological changes.[3,4] The only available medicine in modern practice are cyclooxygenase (COX) inhibitors i.e. NSAIDs and opioids. The use of these classical medicine for long term treatment such as in case of RA, may produces severe adverse effects such as gastrointestinal disturbances, renal damage, respiratory depression, and possible dependence. Therefore, new anti-inflammatory and analgesic drugs lacking those effects are being searched all over the world as alternatives. Medicinal plant have great value to phytochemists because of their medicinal properties[5] so that, the study of plants that have been traditionally used as pain killers should still be seen as a fruitful and logical research strategy in the search for new analgesic drugs and pain mechanisms.[6]

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Bridelia retusa Spreng. *Syn:Bridelia airy shawii* (Family: Euphorbiaceae) is a small to moderate sized deciduous tree, spinous when young with the grey bark, found throughout India up to altitude of 1000 m except in very dry regions. Previous study reported that the bark of *B. retusa* exhibited anti-viral, hypoglycemic and hypotensive properties.[7] According to Ayurveda, the bark is good for removal of urinary concretions, useful in lumbago and hemiplegia. The bark is also used as liniment with gingelly oil in rheumatism[8] (Kirtikar and Basu, 1999). The bark is documented to be used ethno botanically to promote antifertility.

The presence of triterpene ketone [4-desmethyl eupha 7, 24 (28)-diene-3-one] in the bark has been reported. The bark contains 16–40% of tannins.[9] Literature survey revealed that there is no phytopharmacological approach has been made on the *B. retusa* bark. Acute inflammatory effect of *B. retusa* bark has been preliminary investigated by carrageenan-induced hind paw edema.[10] Species of *Bridelia* have been widely used in folk medicine for their rheumatic property.

In general, phytosterols, triterpenoid and tannins have been reported to display anti-inflammatory, anti-ulcer, anti-nociceptive and antiarthritic properties. The objective of the present work was to investigate the analgesic and anti-inflammatory effects of *B. retusa* bark and to elucidate its possible mechanisms of action.

2. Materials and methods

2.1. Plant material and preparation of extracts

The plant material of *B. retusa* bark was collected from Toranmal region of Satpuda hills, India and it was identified by Dr. D.A.Patil, Taxonomist, SSVPS Science College, Dhule, MS, India. A voucher specimen (B-12) of plant was deposited at the RCPCOP herbarium for reference purpose.

The shade-dried bark powder (1 kg) was successively extracted with petroleum ether (60–80 °C) (PE), mixture of methanol: dichloro methane (1:1) and acetone: water mixture (70:30) (ACE). Further residue obtained from methanol: dichloro methane (1:1) was again partitioned with ethyl acetate (EA; 4 × 500 ml). The yields of PE, EA and ACE extracts were 0.7, 1.1 and 9.2% (w/w), respectively. Proximate chemical analysis and pharmacological activity of all the extracts was carried out according to standard method.[11]

2.2. GC-MS analysis

GC-MS analysis of EA was carried out using a gas chromatography with mass (Perkin Elmer USA model auto system XL GC interfaced to a API 20 NL) equipped with a split/split less injector inlet and a flame ionization detector (FID). HP-5MS capillary columns (30 m × 0.25 μm film thickness). The column temperature was programmed at 60 °C (6 min), increasing to 240 °C at a rate of 5 °C/min, carrier gas (helium) was set at a flow rate of 0.9 μL/min; ionization energy 70 eV, and scan mode EI. One μL of sample was injected and the compounds were identified by matching their mass fragmentation pattern and retention time. The compounds were identified by comparison with the mass fragmentation pattern of compounds available in NIST library, USA.

2.3. Separation and isolation of phytoconstituents

The unsaponifiable fraction of PE (5 g) was subjected to column chromatography on a silica gel (60–120 mesh)with gradient elution using petroleum ether: ethyl acetate [(90:10,82:18 v/v) yielded compound 1 (28 mg) and compound 2 (22 mg). Similarly EA fraction was chromatographed over silica gel (60–120 mesh) column, eluted with methanol and chloroform (80:20 v/v) in order of increasing polarity to gave compound 3. All the compounds were identified and characterized by melting point, UV, FT-IR, NMR, HPLC, HPTLC and GC-MS analysis.

2.4. HPLC analysis

The HPLC system of Agilent 1200 series system (Agilent Technologies, Waldbronn, Germany) with photodiode array detector, ODS C18 (5 μm) column was used (150 mm × 4.6 mm) interfaced with an IBM Pentium 4 personal computer. Elution of the phyconstituents with isocratic of two solvents denoted as A and B was employed (Solvent A: acetonitrile and B: water (60:40%), flow rate: 1 ml/min, injection volume: 20 μl, wavelength: 286 nm. The HPLC profile of isolated compounds was compared with reference compound at a specific wavelength. Identification of phytoconstituents was done on the basis of the retention time and identical spectra of standards. The chemical structures of the isolated compounds are given in Fig. 2a–d.

2.5. Experimental animals

Adult albino Wistar rats and Swiss albino mice of either sex weighing between 150–230 and 30–40 g, respectively were used for the study. The animals were housed in well ventilated cages in the air conditioned animal house at 12 h light & dark conditions and fed with standard pellet diet and water *ad libitum*. All the experimental procedure and protocols used in the study were approved by Institutional animal Ethical committee (Protocol number 045/2015) under North Maharashtra University, Jalgaon, India in accordance with Committee for the purpose of Control and supervision on experiments on Animals (CPCSEA), guidelines, Chennai, India.

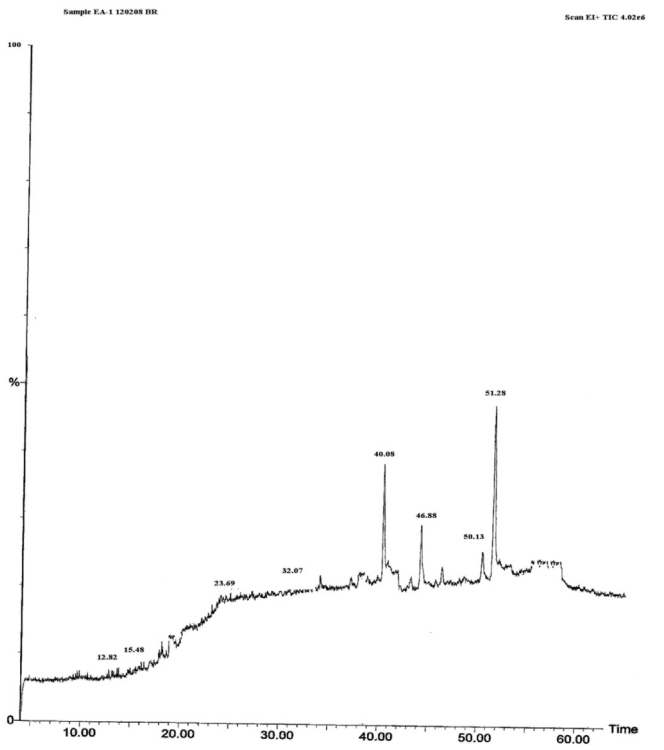

Fig. 1. GC-MS chromatograph profile of EA of *Bridelia retusa* stem bark, Time (min) 40.08: ß-sitosterol; 46.88: stigmasterol; 50.13: lupeol; 51.28 friedelin.

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