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Research paper

Comparative analysis of anti-inflammatory activity of aqueous and methanolic extracts of *Ocimum basilicum* (basil) in RAW264.7, SW1353 and human primary chondrocytes in respect of the management of osteoarthritis

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ABSTRACT

The present study has compared the anti-inflammatory activity of aqueous (OB_W) and methanolic (OB_M) extracts of aerial parts of *Ocimum basilicum* in macrophage (RAW264.7) and human chondrosarcoma (SW1353) cell lines, and human primary chondrocytes to correlate their efficacy in terms of management of osteoarthritis (OA). In RAW264.7, OB_W decreased nitric oxide (NO) (35% ± 0.22) and prostaglandin (PGE_2) (70.8% ± 0.93) production more effectively compared with OB_M. Interestingly, decrease in NO was accompanied by a corresponding decrease in inducible nitric oxide synthase (iNOS) (71.4 ± 2.43%) protein expression. OB_W decreased total nuclear factor-kappa B (NFκB) (79.28% ± 1.8) and cyclooxygenase (COX)-2 (83.87% ± 0.95) proteins significantly ($p < 0.001$), compared with OB_M. Similarly, in SW1353 and chondrocytes, OB_W decreased PGE_2 (76.11% ± 5.5) and leukotriene (LTB4) (59.6% ± 0.22) production appreciably ($p < 0.001$), compared with OB_M. In chondrocytes, OB_W reduced the production of matrix metalloproteinase (MMP)-2 (58.49% ± 1.41), −9 (43.13% ± 2.82) and −13 (54.54% ± 2.12) significantly more ($p < 0.001$), than OB_M. All these data suggest that compared with the methanolic extract, the aqueous extract of *O. basilicum* could be explored for its potential applications in the management of inflammatory conditions associated with OA.

1. Introduction

Osteoarthritis (OA) is a highly prevalent, degenerative joint disease affecting a huge population worldwide (Likivainio et al., 2010). It occurs due to a number of factors (Abramson and Krasnokutsky, 2006; Bonnet and Walsh, 2005) that lead to increase in the production of inflammatory mediators (NO, PGE_2, LTB4, COX-2, MMPs) and pro-inflammatory cytokines (IL-1β, TNF-α) (Ying et al., 2013; Kanouchi, 2013; de Andres et al., 2013; Somma et al., 2013). Even though NSAIDs are widely used to relieve pain and inflammation in OA, their long term use is usually associated with various complications (Raza et al., 2014). Moreover, they lack the potential to prevent continued articular cartilage degeneration (Barron and Rubin, 2007). Thus, recently more attention is being focussed towards the use of traditional medicinal plants as adjunct therapies since they have been used to treat various disease conditions from time immemorial (Wang et al., 2014; Kaul-Ghanekar and Raina, 2012).

Ocimum basilicum (basil), an important medicinal herb, has been traditionally used as an antiseptic, preservative, sedative, digestive regulator and diuretic (Shirazi et al., 2014; Dashputre and Naikwade, 2010). It has also been reported to offer protection from radiation-induced toxic effects (Monga et al., 2011). Various *in vivo* studies have reported anti-inflammatory activity of *O. basilicum* (Rakha et al., 2010; Rameshrad et al., 2014; Benedec et al., 2007; Yadav et al., 2009). The methanolic extract of the whole plant has been shown to exhibit anti-inflammatory activity in peripheral blood mononuclear cells (PBMCs) and RAW264.7 (Selvakkumar et al., 2007). It has also been reported that the methanolic extract of *O. basilicum* leaves provide protection against hepatotoxicity *in vivo* (Saha et al., 2012). Short term administration of a hydroalcoholic leaf extract in rats has been shown to provide protection against myocardial infarction

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(Fathiazad et al., 2012). Moreover, the extract has also been demonstrated to modify the retention and retrieval of memory in mice (Sarahroodi et al., 2012). The aqueous extract of *O. basilicum* leaves has been reported to provide ameliorative effect against deltamethrin induced nephrotoxicity and oxidative stress in albino rats (Sakr and Al-Amoudi, 2012). The extract has also been reported to exhibit immunomodulatory activity. In PBMCS, it has been shown to suppress the production of Th1 (IL-2, IFN-γ, and TNF-β) and Th2 (IL-5, IL-10) cytokines (Tsai et al., 2011). Besides these reports, very few studies have validated its traditional usage, that is, as a potent anti-inflammatory agent (Kamyab and Eshraghian, 2013; Masresha et al., 2012; Woldesellassie et al., 2011; Rahimi et al., 2010). The present research was intended to compare the anti-inflammatory activity of aqueous and methanolic extracts of aerial parts of *O. basilicum* in RAW264.7, SW1353 and human primary chondrocytes in terms of their efficacy to manage OA related pathophysiology. Our results support the claim of traditional usage of *O. basilicum* for the treatment of inflammation. The results revealed that compared to the methanolic extract, the aqueous extract of *O. basilicum* significantly modulated NO, iNOS, PGE_2, LTB4 and MMP levels in RAW264.7, SW1353 and human primary chondrocytes and thus could be explored as a candidate treatment in the management of OA.

2. Methods and materials

RAW264.7 and SW1353 cell lines were purchased from American Type Culture Collection (ATCC, USA), DMEM, L-15 media, Hams F12, FBS, penicillin and streptomycin, Lipopolysaccharide (LPS), IL-1β, dexamethasone, 1400W dihydrochloride and (3-4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma–Aldrich (St. Louis, MO, USA), L-glutamine was purchased from Himedia Corporation, Mumbai, India. Antibodies for NF-κB p65, COX-2 and tubulin were purchased from Santa Cruz Biotechnology, Inc., CA, USA. MMP kit was purchased from Cisbio, PGE_2 and LTB4 kits were purchased from Cayman and tissue culture plasticware was purchased from BD Biosciences (San Diego, CA, USA).

2.1. Plant material and extraction

The extracts of aerial parts of *O. basilicum* (Lamiaceae) were procured from Natural Remedies, Pvt., Ltd., Bangalore that had been collected from Anniyalam, Krishnagiri District, Tamil Nadu, India. The plant material was identified by National Institute of Science Communication and Information Resources (NISCAR), New Delhi and Dr. P. Santhan, in-house taxonomist, Pharmacognosy Department, R&D Centre, Natural Remedies Pvt., Ltd., Bangalore, India. The aerial parts were sun-dried and stored. A voucher specimen (NRPL-569) was deposited in the in-house herbarium of Natural Remedies, Pvt., Ltd., Bangalore.

For the preparation of OB_M, the coarsely powdered raw material (50 g) was extracted with methanol (~200 ml) under reflux at 70 °C for 1 h and the solvent was filtered. The remaining raw material was refluxed by adding 150 ml methanol for 1 h, repeated twice

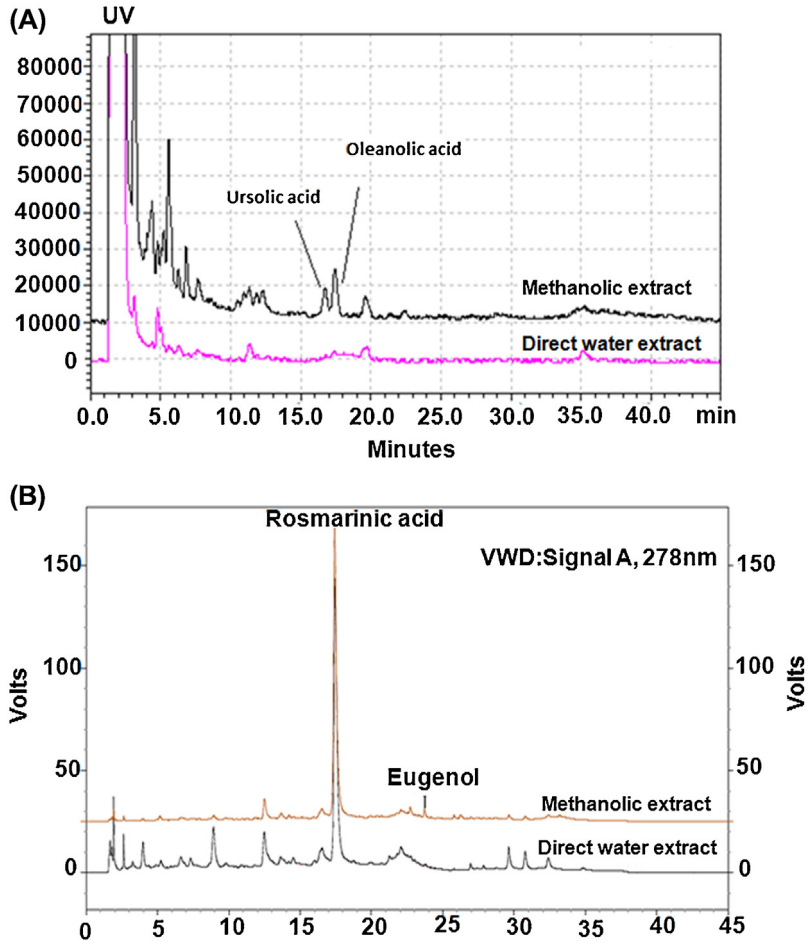

Fig. 1. Phytochemical finger printing of *O. basilicum*.
The constituents of Ocimum species viz., oleanolic acid and ursolic acid (A) as well as rosmarinic acid and eugenol (B) were quantified in aqueous and methanolic extracts by using HPLC.