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Antioxidant and anti-inflammatory properties of marmelosin from Bael (*Aegle marmelos* L.); Inhibition of TNF-α mediated inflammatory/tumor markers

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ABSTRACT

Oxidative stress and inflammation are important critical factors that are implicated in almost all life style disorders such as diabetes, cardiovascular disease, ulcer and cancer. Current study aimed at isolation and characterization of a furanocoumarin from Bael (*Aegle marmelos* L.) fruit that can modulate both oxidative stress and inflammation effectively. Ethyl acetate extract of Bael fruit (EAFB) was subjected to HPLC for identification, purified and characterized using FTIR, NMR and ESI-MS analysis. Predominant peak of EAFB at RT 12.54 min on HPLC was identified as marmelosin with molecular weight of $m/z \sim 271.2$. Marmelosin was evaluated for antioxidant, antiproliferative, apoptotic, cancer (Tyrosinase & Galectin-3) and immunomodulatory (NO, TNF-α) potentials employing standard assay systems. Marmelosin possessed potent antioxidant activity with IC_{50} of ~ 15.4 ± 0.32 μM as opposed to standard - gallic acid (IC_{50} 1.1 ± 0.08 μM), antiproliferative activity with IC_{50} of ~ 6.24 ± 0.16 μM as opposed to deferoxamine (~10.8 ± 0.28 μM) and protected cells against cellular/DNA damage. Anti-inflammatory property was evident with significant reduction in the release of NO (~3.9 fold) and TNF-α (~3.4 fold), a pro-inflammatory cytokine, in addition to the inhibition of NFκB (~2.7 fold), a transcription factor in Raw 264.7 cells. Marked down regulation of galectin-3 (~5.5 folds) and tyrosinase (~11.1 folds) by gene expression analysis substantiated by tyrosinase inhibition (IC_{50} – 20.3 ± 1.26 μM Vs. Kojic acid – IC_{50} – 24.1 ± 1.41 μM) and molecular docking studies strengthened the cancer modulatory property of marmelosin. In addition, marmelosin induced apoptotic bodies, chromatin condensation and nulcear blebbing in Raw 264.7 cells commending the apoptotic effect of marmelosin. Marmelosin thus displayed potential multipotent antioxidant, anti-inflammatory and anticancer properties via TNF-α mediated Akt signaling pathway.

1. Introduction

Oxidative stress (OS) is a condition of exorbitant production of Reactive Oxygen Species (ROS) manifesting the imbalance in oxidants to antioxidants. OS have been known to disturb cellular metabolism leading to cellular/tissue damage and hence disease conditions [1]. Constant productions of ROS are detrimental and play a pivotal role in the development of many degenerative disorders like inflammation, cancer, cardiovascular disorders etc. Inflammation, on the other hand, is a protective physiological immune response against various microbial infections, environmental insults such as carcinogens [2,3]. Recently several reports highlight the role of inflammation in aggravating cancer conditions [3,4].

Macrophages are the major immune cells responsible for detecting and destroying microbial pathogens and apoptotic cells by secreting many bioactive pro-inflammatory mediators, which detects continuous pathogen attack, abnormal ROS generation, persistent secretion of pro-inflammatory mediators at the tissue injury site resulting in dysregulated or abnormal activation of inflammatory signaling pathways. It is important to note that abnormal secretions of pro-inflammatory mediators are mainly responsible for tissue damage, cell transformation and cancer [5]. Suppression of elevated ROS generation, pro-inflammatory mediators and secretion of anti-inflammatory mediators contributes in the positive regulation of dysregulated inflammatory pathways [6,7]. Compounds that have potent ability to fight as both antioxidant and anti-inflammatory agents provide more advantages and in other words,

Abbreviations: AO, acridine orange; BCs, buccal cells; DAPI, 4,6-diaminidino-2-Phenylindole; DMEM, Dulbecco's modified Eagle's medium, DOPA 3,4- dihydroxyphenylalanine; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EtBr, ethidium bromide; LPS, lipopolysaccharide; MTT, (3-(4,5-dimethylthiazol 2-yl)-2,5-diphenyltetrazolium bromide; NO, nitric oxide; RT-PCR, real time polymerase chain reaction; TNF-α, tumor necrosis factor alpha; TdT, terminal deoxynucleotidyl transferase mediated dUTP nick end labeling; UV, ultra violet

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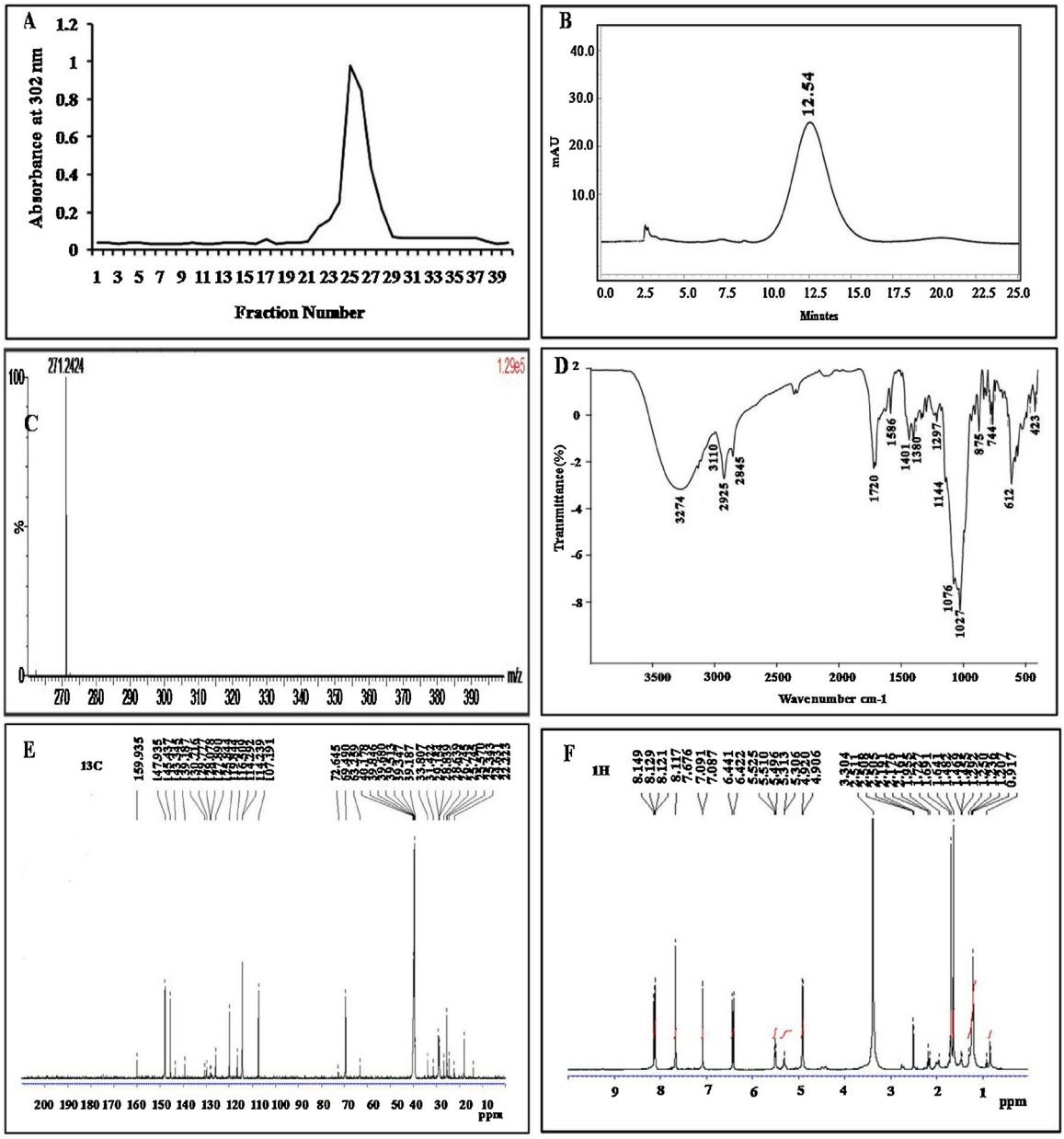

Fig. 1. UV Spectral elution, HPLC, ESI-MS, FTIR and NMR spectral Profiles of Marmelosin.
Fractions eluted from silica gel column was monitored for the emergence of Marmelosin (1A); followed by the determination of homogeneity on HPLC (1 B); Subjected to mass spectral detection by ESI-MS (1 C) and; structural eludication by FTIR (1 D) and NMR spectral (13 C- (1 E), 1H – (1F) analyses.

they can also help to fight against cancer and tissue invasion.

The coumarin (benzopyran-2-one or chromen-2-one) ring system present in natural products that display interesting pharmacological properties, has intrigued chemists and medicinal chemists for decades to explore the natural coumarins or their synthetic analogs for applicability as drugs [8–10] and because of this, many molecules based on the coumarin ring system have been attempted for synthesis utilizing innovative synthetic techniques. The diversity generated in coumarins via synthetic routes led to interesting derivatives including furanocoumarins, pyranocoumarins and coumarin sulfamates (COUMATES), and they were shown to be useful in photo-chemotherapy, anti-tumor/anti-HIV therapy, as stimulants for central nervous system, antibacterial, anti-inflammatory, and anti-coagulants etc. [11,12]. However, there was a concern about their side effects and toxicity [13]. According to FDA, coumarins are “prohibited" since they cause liver and kidney damage [14].

Despite this, the exorbitant health supportive properties of coumarins encouraged pharmaceutical companies to generate semi-synthetic derivatives [15] devoid of undesirable side effects. Current study is therefore attempted to look for yet unexplored natural coumarin derivatives which possess substantial health benefits [16] from Bael. Bael belongs to family Rutaceae, which are known to contain higher levels of furanocoumarins [17,18]. Recently several reports highlight the role of inflammation in aggravating cancer conditions [19]. Investigations were therefore directed towards identification of furanocoumarins in bael and characterization of its biofunctional properties such as antioxidant, immunomodulatory and anticancer properties.

2. Material and methods

2.1. Chemicals

Acridine orange, Ascorbic acid, DAPI, DNA, DOPA, DPPH, Ethidium