Antioxidant and in vitro anti-inflammatory activities of Mimusops elengi leaves

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ABSTRACT

Objective: To assess the antioxidant and in vitro anti-inflammatory activities of the alcoholic extract of Mimusops elengi L. (M. elengi) leaves. Methods: In vitro antioxidant activity was evaluated for peroxynitrite, superoxide and hypochlorous acid scavenging activity. Total phenolic content also determined. Inhibition of protein denaturation and HBRC (Human Red Blood Cell) membrane stabilization method was evaluated for anti-inflammatory activity. Results: The leaf extract of M. elengi exhibited dose dependent free radical scavenging property in peroxynitrite, superoxide and hypochlorous acid models and the IC50 value were found to be (28.93 ± 2.86), (60.5±2.3), (206.4±5.3) μg/mL respectively. Total phenolic content was found to be 97.3 ± 2.7 mg of GAE/g of extract. The maximum membrane-stabilization of M. elengi L was found to be 73.85 ± 0.80% at a dose of 1 000 μg/mL of extract and that of protein denaturation was found to be 60.23% at a dose of 290 μg/mL. Conclusion: From the result it can conclude that M. elengi extract showed good antioxidant and in vitro anti-inflammatory activities.

Keywords: Mimusops elengi Total phenolic content Antioxidant activity Anti-inflammatory activity HBRC membrane stabilization

1. Introduction

Free radical is defined as unstable, highly reactive atom or molecule possessing unpaired electrons, which induces free radical damage. Reactive oxygen species (ROS) are widely reactive oxygen species (ROS) are widely produced in living systems and can exert toxic or physical, chemical or traumatic damage[2]. One purpose of inflammation is to protect the site of an injury. The results were compared with diclofenac sodium (250 μg/mL).

2. Materials and methods

2.1. Collection and extraction of plant material

The fresh leaves of M. elengi were collected from Cuttack (District of Odisha) and identified by Dr Mondal, taxonomist scientist explored for its anti-inflammatory activity. The bark of M. elengi has been used for antioxidant activity[5], antibacterial[6], and applied to the head as a cold compress for headache[4]. The chemicals used were Biovine serum albumin (BSA), Folin-Coloral (FC) reagent, Ascorbic acid, Diclofenac sodium was purchased from Sigma-Aldrich. All other chemicals and reagents used were of highest analytical grade.

2.2. Drugs and chemicals

The control solution (0.5 mL) consisted of 0.45 μL of distilled water and 0.05 mL of distilled water. The scavenging activity was measured against distilled water as blank. Total phenolic content was found to be 97.3 ± 2.7 mg of GAE/g of extract. The maximum membrane-stabilization of M. elengi L was found to be 73.85 ± 0.80% at a dose of 1 000 μg/mL of extract and that of protein denaturation was found to be 60.23% at a dose of 290 μg/mL with regards to standards in the antioxidant activity. From the result it can conclude that M. elengi extract showed good antioxidant and in vitro anti-inflammatory activities.

2.3. Determination of total phenolic content

Total phenolic content was determined using Folin-Ciocalteu (FC) reagent according to the reference method[3] with slight modification. Briefly 1 mL (1 mg) of extract, 45 μL of distilled water and 1 mL FC were mixed in a conical flask, and was shaken for 30 min. Then 3 mL of Na2CO3 (2%) were added to the mixture and shaken for 2 h at room temperature. The absorbance was measured at 760 nm against distilled water as blank. Total phenolic content was calculated according to the following formula.

Absorbance at 760 nm = 0.001 × Pyrocatechol (μg) + 0.003 3

2.4. Antioxidant activity

2.4.1. Peroxynitrite scavenging

The scavenging activity was measured by using an Evans Blue bleaching assay. 1 mL reaction mixture contained 50 mM phosphate buffer (pH 7.4), 0.1 mM DTPA, 50 mM NaCl, 5 mM KC1, 2.5 mM Evans blue. Different concentration of extract or standard ascorbic acid and 1 mM freshly prepared peroxynitrite. The absorbance was measured after 30 min of incubation at 570 nm. The concentration of ONOO− was measured spectrophotometrically at 302 nm (E = 1.670 M−1 cm−1). The percentage scavenging of ONOO− was calculated by using the following formula.

Percentage inhibition = (Absorbance of control - Absorbance of test) / Absorbance of control × 100

2.4.2. Superoxide radical scavenging

Reaction mixture contains 1 mL of nitroblue tetrazolium (NBT, 156 ± 3 μL), 1 mL of reduced nicotinamide adenine dinucleotide (NADH, 468 ± 3 μL) and 3 mL of the test solution or standard ascorbic acid at different concentrations. The reaction was initiated by adding 100 μL of pherase methysulphanphate (PMS, 60 ± 3 μL) and incubated at 25 °C for 10 min. All the solutions were prepared in 100 mM phosphate buffer (pH 7.4). Then absorbance was measured at 560 nm and the scavenging reaction was calculated by using the above mentioned formula.

2.4.3. Hypochlorous acid scavenging

Hypochlorous acid (HOCI) was freshly prepared adjusting the pH of a 10g/v solution of NaOCl to 6.2 with 0.6 M H2SO4, and the concentration of HOCI was determined by measuring the absorbance at 235 nm using the molar extinction coefficient of 100 M−1 cm−1. The reaction mixture contained, 1.5 mL of HOCI and different concentration of the extract or standard ascorbic acid and incubated for 1 h at 37 °C. After that taurine (30 mM) was added and incubated again 30 min at 37 °C followed by the addition of thiostrin benzoic acid (TNB). Absorbance was measured at 412 nm against blank and was scavenging was calculated according to the standard formula.

2.5. In vitro anti-inflammatory activity

2.5.1. Inhibition of protein denaturation method

Test solution (0.5 mL) consist of 0.45 μL of BSA (5% w/v aqueous solution) and 0.05 mL of test solution (250 μg/mL). Control solution (0.5 mL) consisted of 0.45 μL of distilled water and 0.05 mL of distilled water. The scavenging activity was measured against distilled water as blank. Total phenolic content was found to be 97.3 ± 2.7 mg of GAE/g of extract. The maximum membrane-stabilization of M. elengi L was found to be 73.85 ± 0.80% at a dose of 1 000 μg/mL of extract and that of protein denaturation was found to be 60.23% at a dose of 290 μg/mL. Conclusion: From the result it can conclude that M. elengi extract showed good antioxidant and in vitro anti-inflammatory activities.

2.5.2. HBRC membrane stabilization method

The principle concerned in this method is stabilization of human red blood cell membrane by hypo toxicity induced membrane lysis. Blood was collected (2 mL) from healthy volunteers and was mixed with equal volume of sterile Alsevers solution (2% dextrose, 0.8% sodium citrate, 0.9% sodium chloride, 0.42% NaCl in distilled water and centrifuged at 3 000 rpm. The packed cells were washed with isosalone

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solution and a 10% v/v suspension was prepared with normal saline and kept at 4 °C undisturbed before use. Different concentrations of M. elengi extract (50, 100, 200, 500 and 1 000 μg/mL) in normal saline, dichlofenac sodium as standard (50, 100, 200, 500 and 1 000 μg/mL) and control distilled water instead of hyposaline to produce 100% hemolysis were separately mixed with 1 mL of phosphate buffer, 2 mL of hyposaline and 0.5 mL of 10% HBSS suspension was added to prepared. All the assay mixtures were incubated at 37 °C for 30 min and centrifuged at 2 000 rpm for 20 min and hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm [17]. The percentage of HBBC membrane stabilization or protection was calculated by using the following formula:

\[ \text{Percentage Stabilization} = \frac{\text{Absence of control} - \text{Absence of Test}}{\text{Absence of control} - \text{Absence of control}} \times 100 \]

### 2.6. Statistical analysis

The experimental data were expressed as mean ± SEM. The inhibitory concentrations 50% (IC50) were calculated by plotting the data in the graph as concentration versus percentage inhibition using GraphPad Prism software, version 5.

### 3. Result

#### 3.1. Total phenol contents

Total phenolic content was determined using the Folin–Ciocalteau (FC) reagent and it was calculated as 97.3 μg/mg of M. elengi extract which is accounted for its free radical as well as antioxidant activity.

#### 3.2. Antioxidant activity

##### 3.2.1. Peroxynitrite scavenging

The peroxynitrite scavenging activity was found (Figure 1) in a dose dependent manner as compared to standard ascorbic acid. The IC50 values (Figure 2) were found to be (205.3 ± 2.3) μg/mL and (56.8 ± 2.3) μg/mL for M. elengi extract and standard respectively.

##### 3.2.2. Superoxide radical scavenging

Superoxide radicals generated from dissolved oxygen by PMS±NADH coupling may be measured by an ability to reduce NBT. The increase in inhibition capability indicates the extract has good superoxide radicals scavenging activity as compared to standard ascorbic acid (Figure 3). The IC50 values of M. elengi extract and ascorbic acid were found to be (60.5 ± 3.5) μg/mL and (8.61 ± 1.3) μg/mL (Figure 4) respectively.

##### 3.2.3. Hypochlorous acid scavenging

Dose-dependent hypochlorous acid scavenging activity of M. elengi extract and standard ascorbic acid was found in this study (Figure 5). The IC50 values (Figure 6) of extract and standard were found to be (202.4 ± 5.3) μg/mL and (190.47 ± 5.23) μg/mL respectively.

### 3.4. In vitro anti-inflammatory activity

#### 3.4.1. Inhibition of protein denaturation method

The M. elengi extract showed maximum inhibition of protein denaturation of 96.23 ± 0.8% at 250 μg/mL and its effect was compared with the standard anti inflammation drug, dichlofenac sodium showed the maximum inhibition (94.22 ± 0.30%) at the same concentration.

#### 3.4.2. In vitro anti-inflammatory activity

In the in vitro anti-inflammatory activity screening it was observed that the M. elengi extract showed protective activity when compared to the standard dichlofenac sodium. The percentage of stabilization was found to be 73.85 ± 0.8% and 94.23 ± 0.5% at concentration of 1 000 μg/mL of M. elengi extract and standard respectively. The activity of the extracts were concentration dependent, with the increasing concentration the activity is also increased. These results may be attributed due to the presence of phenolic content, good antioxidant properties.

### 4. Discussion

The data presented in this study demonstrate that M. elengi extract possess antioxidant and in vitro anti-inflammatory activity. Indeed, M. elengi extract scavenged peroxynitrite, superoxide and hypochlorous acid in a concentrated dependent manner.

Peroxynitrite (ONOO−) is relatively stable and toxic compound compared to other free radicals but once protonated it forms the highly reactive peroxynitrite acid (ONOOH). Generation of excess ONOO− leads to oxidative damage and tissue injury[16]. According to the present results, the M. elengi extract inhibits evans blue bleaching by scavenging peroxynitrite.

The most important ROS produced by the inflammatory cells is superoxide, hydrogen peroxide, hydroxyl radical and hypochlorous acid. Membrane bound NADPH oxidase reduce the molecular oxygen to produce the superoxide anions which interm converted to hydrogen peroxide and hydroxyl radical as well as hypochlorous acid by using different enzymatic reaction in our body[15]. In this present study, the M. elengi extract exhibited concentration-dependant increasing of superoxide radical scavenging activity.

At sites of inflammation, the oxidation of Fe3+ ions by the neutrophil enzyme myeloperoxidase results in the production of another harmful HOCl which have the ability to react with the tissue and the resultant compound decrease the color of TNB[30], M. elengi extract showed dose dependent inhibition of HOCl.

These observed antioxidant activities suggest that the alcoholic extract M. elengi extracts was found to possess concentration dependent scavenging activity on peroxynitrite, superoxide and hypochlorous acid. It could exert protective effects also in vivo against oxidative and free radical injuries occurring in different pathological conditions, which may be potentially responsible for its anti-inflammatory activity.

The presence of high phenolic content in the M. elengi extract has been observed by FC reagent test and also from phytochemical test, it was also found as a good source of flavonoids and triterpenes. Therefore the anti-inflammatory activity of M. elengi extract of seems to be due to the phenic compounds in it[31].

Denaturation of protein is one the cause of denaturation. The production of auto antigens in inflammation disease may be due to in vivo denaturation of protein. The mechanism of denaturation possibly involves alteration in electrotetric, hydrogen, hydrophobic and disulphide bonding[32]. From the result, it can be stated that the extracts of M. elengi was capable of controlling the production of auto antigen and thereby it inhibit the denaturation of proteins and its effect was compared with the standard drug dichlofenac sodium.

M. elengi L. leaves extracts exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The erythrocyte membrane is analogous to the liposomal membrane[33] and its stabilization implies that the extract may as well stabilize lyosomal membranes. Stabilization of lysosomal membrane is important in the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bacterial enzymes and proteases.
which cause further tissue inflammation and damage upon extra cellular release[24]. Though the exact mechanism of the membrane stabilization by the extract is not known yet; hypotonicity-induced hemolysis may arise from shrinkage of the cells due to osmotic loss of intracellular electrolyte and fluid components or interaction with membrane proteins[25,26]. The extract may inhibit the processes, which may stimulate or enhance the efflux of these intracellular components. On the basis of the above results it can be concluded that the *M. elengi* extracts have an anti-inflammatory activity.

The present investigation has shown that the alcoholic extract of *M. elengi* exhibited concentration dependent free radical scavenging activity. These activities due to strong occurrence of phenolic compounds such as flavonoids, tannins, terpenoids, phenols and saponins. This antioxidant effect may be responsible for *in vitro* anti-inflammatory activity of the alcoholic extract of *M. elengi*. Studies are in progress in order to isolate and identify some active compounds which might be responsible the activity, also to understand the exact mechanism of action in relation to the observed anti-inflammatory activity.

**Conflict of interest statement**

We declare that we have no conflict of interest.

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**Reference**


