ANTIOXIDANT ACTIVITIES AND PHENOLIC CONTENTS OF EXTRACTS FROM GLINUS OPPOSITIFOLIUS AERIAL PARTS

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ANTIOXIDANT ACTIVITIES AND PHENOLIC CONTENTS OF EXTRACTS FROM GLINUS OPPOSITIFOLIUS AERIAL PARTS (Abstract): In Malian traditional medicine, aerial parts of Glinus oppositifolius are used in the treatment of many disorders, particularly inflammations and joint pains. **Aim:** The present study was initiated to investigate the antioxidant potential of Glinus oppositifolius. **Material and methods:** Aqueous and ethanol extracts were prepared from the dried aerial parts. Both extracts were examined for the phenolic content and antioxidant activity. Total phenolic content was quantified by Folin-Ciocalteu method. Antioxidant activity was studied in vitro by evaluating the ability to scavenge different free radicals (ABTS, superoxide and hydroxyl radicals, nitric oxide) and to chelate ferrous ions. **Results:** Aqueous extract was found to contain a higher level of total phenols than ethanol extract (1.27±0.04 vs. 0.94±0.05 g GAE/100 g extract). Both extracts lacked the ability to scavenge superoxide anion radical but scavenged ABTS and hydroxyl radicals, nitric oxide and chelated ferrous ions in a concentration-dependent manner. According to the EC50 values, aqueous extract showed stronger antioxidant effects than ethanol extract (174.32±1.49 vs. 276.76±1.52 µg/mL in ABTS radical cation scavenging assay, 119.91±1.70 vs. 240.57±0.97 µg/mL in nitric oxide scavenging assay, 1.99±0.01 vs. 6.54±0.08 mg/mL in ferrous ion chelating assay). In hydroxyl radical scavenging assay, aqueous extract had an EC50 value of 0.86±0.00 mg/mL while the EC50 value of ethanol extract was higher than the highest concentration that was tested (3.75 mg/mL). **Conclusions:** Our results clearly indicate the antioxidant potential of Glinus oppositifolius aerial parts and suggest that the anti-inflammatory activity might be partly related to the antioxidant potential. **Keywords:** Glinus oppositifolius, INFLAMMATION, OXIDATIVE STRESS, FREE RADICAL SCAVENGING ACTIVITY, FERROUS ION CHELATING ACTIVITY

Glinus oppositifolius (L.) Aug. DC. (Aizoaceae) is native to tropical and subtropical regions of the world, its leaves and aerial parts being used either as food or medicine in different ailments. In Malian traditional medicine the plant has been used in the treatment of joint pains, inflammations, fever, skin disorders, wounds, urinary
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Infections, abdominal pains, diarrhoea, intestinal parasitosis, malaria. In an attempt to valorize Malian traditional medicines, researchers from School of Pharmacy, University of Oslo and Department of Traditional Medicine, Bamako, Mali carried out chemical and biological studies on the aerial parts of the species. Triterpenoid saponins and pectic polysaccharides have been isolated and characterized. Pectic polysaccharides showed immuno-modulating activity by fixing the complement system components and increasing the chemotaxis of macrophages, NK cells and T cells (1-3).

*Glinus oppositifolius* is one of the herbs Malian traditional healers use in the treatment of inflammations and joint pains. In addition to an enhanced immune response, inflammation is characterized by an increased oxidative stress (4, 5). The aim of the present study was to evaluate a possible antioxidant activity involved in the anti-inflammatory potential of *Glinus oppositifolius*. In this respect, the antioxidant activity of aqueous and ethanol extracts was studied by evaluating their ability to scavenge free radicals and to chelate ferrous ions. The total phenolic content was also determined.

**MATERIAL AND METHODS**

**Chemicals.**

Tris(hydroxymethyl)aminomethane (Tris), ferrous chloride, sodium nitroprusside were purchased from Merck (Darmstadt, Germany). 2, 2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), ethylenediaminetetraacetic acid (EDTA), 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4',4''-disulphonic acid monosodium salt (ferrozine), glutathione, pyrogallol, Folin-Ciocalteu's phenol reagent were supplied by Fluka (Steinheim, Germany). Iron (II) sulfate heptahydrate, hydrogen peroxide, L-ascorbic acid, N-(1-naphthyl) ethylenediamine dihydrochloride, sodium salicylate, sulfanilamide, were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Gallic acid monohydrate and potassium persulphate were purchased from Riedel-de Haën (Seelze, Germany).

**Plant material.** Aerial parts of *Glinus oppositifolius* (L.) Aug. DC. were supplied by the Department of Traditional Medicine, Bamako, Mali where a voucher specimen is deposited.

**Extraction.** Dried and powdered aerial parts (100 g) were refluxed with 2 × 2 L ultrapure water at 50°C, each time for 4 h or extracted by stirring with 2 × 2 L ethanol at room temperature, each time for 72 h. The combined aqueous and ethanol extracts were concentrated under reduced pressure at 50°C (Büchi R-210 rotary evaporator, Büchi V-850 vacuum controller, Büchi V-700 vacuum pump, Büchi B-490 heating bath) and then freeze-dried (Unicryo TFD 5505 freeze-dryer).

**Total phenolic content.** Total phenolic content was estimated by Folin-Ciocalteu method (6). The results were expressed as g gallic acid equivalents (GAE)/100 g extract.

**ABTS radical cation scavenging assay.** ABTS assay was performed according to the method developed by Re et al. (7). Glutathione was the positive control.

**Superoxide anion radical scavenging assay.** The assay was performed as described by Wang and Luo (8). Glutathione was used as positive control.

**Hydroxyl radical scavenging assay.** The assay was done according to the method described by Jeong et al. (9). L-Ascorbic
acid was used as positive control.

**Nitric oxide scavenging assay.** The assay was performed as reported by Tsai *et al.* with minor changes (10). L-Ascorbic acid was the positive control.

**Ferrous ion chelating ability assay.** The assay was done according to Dinis *et al.* (11). EDTA was used as positive control.

**Statistical analysis.** All experiments were performed in triplicate. The results were expressed as means±standard deviations. The EC$_{50}$ values were calculated by linear interpolation between values above and below 50% activity.

**RESULTS**

**Extraction.** After removing the solvents, 40 g aqueous extract (GA) and 23.7 g ethanol extract (GE) were obtained.

**Total phenolic content.** The total phenolic contents of GA and GE were found to be 1.27±0.04 and 0.94±0.05 g GAE/100 g, respectively.

**ABTS radical cation scavenging assay.** Both extracts scavenged ABTS radical cation in a concentration dependent manner. At 100 µg/mL, GA and GE showed scavenging effects of 35.42±0.47 and 27.37±0.66%, respectively. At 400 µg/mL, the scavenging activity of GA and GE increased significantly to 73.75±0.15 and 65.12±1.01%, respectively. Within the same concentration range (100-400 µg/mL), glutathione completely scavenged the radical (99.47±0.29-99.68±0.02%) (fig. 1). According to the EC$_{50}$ values, the abilities to scavenge ABTS radical cation varied as follows: glutathione > GA > GE (tab. I).

**Superoxide anion radical scavenging assay.** Within this assay, GA and GE showed no scavenging effects on superoxide anion radical.

**Hydroxyl radical scavenging assay.** GA showed a higher hydroxyl radical scavenging activity than GE. At the highest concentration tested (3.75 mg/mL), GA scavenged the radical in a percentage of 91.69±0.09% while GE showed only 31.33±0.74% scavenging activity. GA was less active than the positive control, L-ascorbic acid; at concentrations of 0.47-3.75 mg/mL, the latter exhibited 100% scavenging activity (fig. 2). With regard to the EC$_{50}$ values, the hydroxyl radical scavenging activity decreased as follows: L-ascorbic acid > GA > GE (tab. I).

**Nitric oxide scavenging assay.** Both extracts showed dose-dependent nitric oxide scavenging effects. The scavenging activity of GA increased significantly from 42.10±0.57% at 83.33 µg/mL to 83.47±0.17% at 1333.36 µg/mL. At the same concentrations, GE exhibited scavenging effects of 22.37±0.70 and 86.59±0.46%, respectively. The effects of L-ascorbic acid were superior to those of GA and GE at all concentrations tested (fig. 3). According to the EC$_{50}$ values, the nitric oxide scavenging effects decreased in the following order: L-ascorbic acid > GA > GE (tab. I).

**Ferrous ion chelating ability assay.** Ferrous ion chelating abilities of both extracts increased with concentration. At all concentrations tested, the chelating ability of GA was greater than that of GE but lower than that of EDTA. At the highest concentration tested (6.62 mg/mL), the chelating effects of GA and GE were 76.53±0.48 and 50.40±0.75%, respectively; at the same concentration, EDTA completely chelated the ferrous ions (fig. 4). The ferrous ion chelating ability decreased as follows: EDTA > GA > GE (tab. I).
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Fig. 1. ABTS radical cation scavenging activity

Fig. 2. Hydroxyl radical scavenging activity

Fig. 3. Nitric oxide scavenging activity
Table I

Antioxidant activities of *Glinus oppositifolius* (L.) Aug. DC. aerial part extracts

<table>
<thead>
<tr>
<th>Extract/ Positive control</th>
<th>EC$_{50}$ ABTS radical cation scavenging activity</th>
<th>EC$_{50}$ Hydroxyl radical scavenging activity</th>
<th>EC$_{50}$ Nitric oxide scavenging activity</th>
<th>EC$_{50}$ Ferrous ion chelating activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA</td>
<td>174.32 ± 1.49*</td>
<td>0.86 ± 0.00*</td>
<td>119.91 ± 1.70*</td>
<td>1.99 ± 0.01**</td>
</tr>
<tr>
<td>GE</td>
<td>276.76 ± 1.52*</td>
<td>&gt; 3.75*</td>
<td>240.57 ± 0.97*</td>
<td>6.54 ± 0.08**</td>
</tr>
<tr>
<td>Glutathione</td>
<td>3.35 ± 0.01*</td>
<td>&gt; 3.75*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L-Ascorbic acid</td>
<td>-</td>
<td>0.12 ± 0.00*</td>
<td>45.56 ± 0.45*</td>
<td>-</td>
</tr>
<tr>
<td>EDTA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6.3 ± 0.0*</td>
</tr>
</tbody>
</table>

GA – aqueous extract; GE – ethanol extract
* results expressed in μg/mL; ** results expressed in mg/mL

**DISCUSSION**

Numerous studies have reported the involvement of reactive oxygen species in inflammation. After infiltration to the inflammatory site, polymorphonuclear leukocytes and macrophages release high levels of superoxide anion radical and hydrogen peroxide (respiratory burst). Superoxide anion radical is able to release iron from ferritin as ferrous ions. Ferrous ions promote the generation of hydroxyl radical via Fenton reaction. Hydroxyl radical is the most aggressive reactive oxygen species being responsible for protein, lipid and DNA damage (4, 5, 12). In inflammation, inducible nitric oxide synthase (iNOS) generates high levels of nitric oxide. Nitric oxide enhances the production of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNFα), interleukin-1β (IL-1β), but also of collagenases thus promoting the progression of inflammation (12). Nitric oxide in excess generates enhanced levels of reactive nitrogen species which target cellular macromolecules causing additional tissue damage (10).

This study indicated that *Glinus oppositifolius* extracts have antioxidant activity. GA and GE scavenged free radicals such as hydroxyl radical and nitric oxide and chelated ferrous ions. GA was found to be more active than GE. The stronger antioxidant activity of GA cannot be justified solely by its higher phenolic content; other
phytochemicals may also contribute to the antioxidant potential of GA. GA scavenged hydroxyl radicals and chelated ferrous ions with EC$_{50}$ values of 0.86±0.00 and 1.99±0.01 mg/mL, respectively; in the same assays, GE showed weaker effects (EC$_{50}$ > 3.75 mg/mL, EC$_{50}$ = 6.54±0.08 mg/mL). In nitric oxide scavenging assay, GA (EC$_{50}$ = 119.91±1.70 µg/mL) was only 2.6 times less active than L-ascorbic acid (EC$_{50}$ = 45.46±0.45 µg/mL). As L-ascorbic acid is a strong nitric oxide scavenger (13), it is obvious that GA exhibited a very good scavenging activity. The high scavenging activity against nitric oxide, the ability to eliminate hydroxyl radicals and chelate ferrous ions make GA a good candidate for in vivo studies. Neither GA nor GE scavenged superoxide anion radicals. The lack of superoxide scavenging effects is surprising given that both extracts scavenged other free radicals. It is possible that certain phytochemicals in extracts act as prooxidants under the conditions of this assay thus masking the superoxide scavenging activity. It is worthy to note that there have already been reported prooxidant effects for certain flavonoids, alphatocopherol, coenzyme Q10, clove essential oil and butylated hydroxytoluene when tested in this assay (14, 15).

**CONCLUSIONS**

It can be concluded that the anti-inflammatory potential of *Glinus oppositifolius* is partly related to its antioxidant properties.

Further studies are needed to evaluate the in vivo significance of the antioxidant activity and its role in the regression of inflammation.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


CEREBROPROTECTIVE EFFECTS OF IBUPROFEN ON DIABETIC ENCEPHALOPATHY IN RATS

A group of researchers from Xuzhou Medical College and Nanjing General Hospital of Nanjing Military Command, Jiangsu, China investigated the effects of ibuprofen on diabetic encephalopathy and the potential mechanisms. Diabetes was induced through a single intraperitoneal injection of streptozotocin (60 mg/kg). The diabetic rats were randomly divided into two groups: the control and the treated groups. The treatment was initiated 7 days after induction of diabetes and consisted in oral administration of ibuprofen (40 mg/kg) for 8 weeks. Cognitive performances were evaluated using Morris water maze. The temporal cortex and hippocampus were obtained to evaluate the levels of advanced glycation endproducts (AGEs) and their receptor (RAGE), the activity, protein expression, and mRNA levels of β-amylloid precursor protein cleaving enzyme 1 (BACE1), the protein and mRNA expression of peroxisome proliferator-activated receptor γ (PPARγ), and the protein expression of cyclooxygenase 2 (COX-2) and inducible nitric oxide synthase (iNOS). Blood was obtained for the evaluation of interleukin 1β level. The research revealed that chronic ibuprofen treatment reduced the mean escape latency of diabetic animals and significantly prevented the decline in learning and memory ability of diabetic rats. Ibuprofen treatment recovered the enzymatic activity of BACE1 to the control level in the temporal cortex, while markedly reduced enzymatic activity in the hippocampus of diabetic rats. Moreover, ibuprofen treatment reduced the activity, protein, and mRNA levels of AGE level, protein expression of RAGE, COX-2, and iNOS in the brain, and interleukin 1β level in serum, while increasing the protein and mRNA expression of PPARγ in the brain of diabetic rats. The present study demonstrated that ibuprofen ameliorated diabetic encephalopathy caused by type 1-diabetes, as evidenced by the improvement of cognitive dysfunction and the loss of neurons in the hippocampus. Furthermore, the amelioration of diabetic encephalopathy by ibuprofen was achieved by the down-regulation of BACE1, the suppression of the AGE/RAGE axis and inflammation in the brains of diabetic rats. (Liu Y-W, Zhu X, Zhang L, Lua Q, Zhang F, Guoa H, Yin X-X, Cerebroprotective effects of ibuprofen on diabetic encephalopathy in rats. Pharmacol Biochem Behav 2014; 117: 128-136)