



Anti-inflammatory and antioxidant activities of *Sclerochloa dura* (Poaceae)

SYED MAJID BUKHARI^{1*}, ASTRID JULLUMSTRØ FEUERHERM², FAYSSAL BOULFRAD³, BOJAN ZLATKOVIĆ⁴, BERIT JOHANSEN² and NEBOJŠA SIMIĆ¹

¹Department of Chemistry, Norwegian University of Science and Technology, 7491 Trondheim, Norway, ²Department of Biology, Norwegian University of Science and Technology, 7491 Trondheim, Norway, ³UFR de Pharmacie, Grenoble, France and ⁴Faculty of Sciences, University of Niš, 18000 Niš, Serbia

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Abstract: The plant *Sclerochloa dura* is traditionally used in South-East Serbia to treat menstrual disorders characterized by pain and excessive bleeding. According to statements of subjects, a reduction in bleeding and pain is experienced shortly after oral intake. The focus of this investigation was to determine the inhibitory effects of the plant on the arachidonic acid (AA) cascade along with the spectrophotometric determination of its antioxidant capacity. The AA release assay was performed using the human fibroblast-like synovio-cyte cell line SW982 to determine the AA release and hence phospholipase A₂ (PLA₂) activity. The crude extract and subsequent fractions of *S. dura* inhibit IL-1 induced release of AA in a time- and dose-dependent manner in SW982 cells. The IC₅₀ for the crude extract is 1.5 mg mL⁻¹ at 4 and 24 h of stimulation. Treating the cells with 0.22, 0.11 and 0.06 mg mL⁻¹ of a methanolic fraction resulted in 97, 91 and 63 % inhibition of AA-release, respectively. One milligram of the crude extract contained 34.78 µg pyrocatechol equivalent phenolic content, 22.80 µg quercetin equivalent flavonoid content and an antioxidant activity of 70.11 µg α-tocopherol equivalents. The strong inhibitory effects of the *S. dura* extracts on the AA cascade may explain the reported pain- and discomfort-relieving effects.

Keywords: arachidonic acid release assay; cytosolic phospholipase A₂ enzyme; flavonoid content; free radical scavenging activity; phenolic content; SW 982 fibroblast-like synoviocytes.

INTRODUCTION

Eicosanoids, such as prostaglandins and leukotrienes, are derivatives of the ω-6 fatty acid (AA) and act as potent lipid mediators of inflammation.¹ AA is released by the action of phospholipase A₂ (PLA₂) enzymes by hydrolysis of the

*Corresponding author. E-mail: majid_bukhari@hotmail.com
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sn-2 ester bond of membrane glycerophospholipids. PLA₂ enzymes are primarily sorted into five categories; secretory PLA₂ (sPLA₂), cytosolic PLA₂ (cPLA₂), Ca²⁺-independent PLA₂ (iPLA₂), platelet-activating factor acetyl hydrolases (PAF-AH) and lysosomal PLA₂s.^{2,3} Alongside the ongoing elucidation of the roles of different PLA₂ isotypes in physiology or pathophysiology of different diseases,⁴ there is great interest in the development of different PLA₂ subtype-specific inhibitors to treat human diseases.⁵

Pro-inflammatory cytokines, such as TNF and IL-1 β , activate PLA₂ enzymes, mainly the arachidonyl specific cPLA₂-IV α isotype, resulting in the subsequent release of AA and the generation of pro-inflammatory eicosanoids.⁶⁻⁸ In addition, it was previously shown that activated TNF and IL-1 β may lead to increased transcription of the cyclooxygenase 2 (COX2) and cPLA₂-IV α genes, further propagating inflammation by increased availability and metabolism of AA into pro-inflammatory eicosanoids.^{8,9} In addition to their association with inflammation, increased availability of AA has also been connected with heavy menstrual bleeding.¹⁰ Prostaglandins, such as PGE₂ and PGF₂, levels have been found to be elevated in the endometrium of women with heavy menstrual bleeding compared to women with normal menses.^{11,12} Prostaglandins contribute to uterus contractions¹³ and are thought to be a major factor in primary dysmenorrhea. By targeting the cyclooxygenases (COX1/2) responsible for the enzymatic conversion of AA to eicosanoids, non-steroidal anti-inflammatory drugs are effective in relieving the pain and discomfort of dysmenorrhea.¹¹

A good anti-inflammatory activity often accompanies good antioxidant activity.^{14,15} There are two basic types of antioxidants available: synthetic and natural ones. The synthetic antioxidants typically contain phenolic groups as the main functionality. The natural antioxidants are mostly obtained from different parts of plants and their structural diversity is much larger. They can be either nitrogen-containing compounds, such as alkaloids, chlorophyll derivatives, amines and amino acids, or phenolic compounds, such as tocopherols, flavonoids and phenolic acids. These compounds can act as oxygen scavengers, thereby terminating the harmful activity of free radicals.^{16,17} Flavonoids are considered to be the most potent antioxidants. They can delay or inhibit the oxidation of lipids or other molecules by inhibiting the propagation of oxidative chain reactions.¹⁸ There has been an increased interest in natural antioxidants from plant materials in the recent years.¹⁹

Sclerochloa dura (Linnaeus) P. Beauvois, also known as common hardgrass, belongs to family Poaceae Barnhart, which involves more than 700 genera and almost 50,000 species. It is an annual plant, with flat leaves and a procumbent or erect stem. The inflorescence is a crowded, one-sided series of flattened spikelets.²⁰ The plant is a common inhabitant of areas with heavy traffic, *e.g.*, along dirty roads, on play yards and walking pathways. It is widely spread in the mode-

rate climate zone on almost all the continents of the North Hemisphere and was introduced to Australia as well.²¹

There are only a few published papers in which this plant is mentioned. However, none of them deal with its chemical composition or bioactivity. In most of the studies, the plant was used as a specimen for the determination of the efficiency of various herbicides.^{22–28} Two papers discuss the phylogeny of certain grasses, among them *S. dura*^{29,30} and one paper deals with the identification of prolamines in cereal and grass species, including *S. dura*.³¹

The plant is traditionally used in South-East Serbia for the treatment of menstrual disorders, manifested as excessive bleeding and intense pain. Professor Aleksandar Igić (Medical faculty, University of Niš, Serbia) in personal correspondence reported that in this region tea or decocts of *S. dura* have been used by women having menstrual disorders. According to the statements of subjects, the symptoms were significantly alleviated shortly after intake. The remarkable story about the health improving properties of *S. dura*, together with the lack of published data about its chemical composition and bioactivity, was the reason to start studying this plant. Therefore, in this study, the aim was to determine the chemical composition, including free radical scavenging activity, anti-oxidant activity, the total phenolic and flavonoid contents of *S. dura* extract, and its ability to inhibit the release of AA. In addition, such properties of *S. dura* were compared with those reported for other plants reported to alleviate menstrual disorders, *i.e.*, *Wrightia tomentosa*^{32,33} and *Dendrophthoe falcata*.³⁴ The novel findings presented herein, forward the plant *S. dura* as a promising natural source for alleviating inflammatory disorders, including menstrual discomfort.

EXPERIMENTAL

Plant material

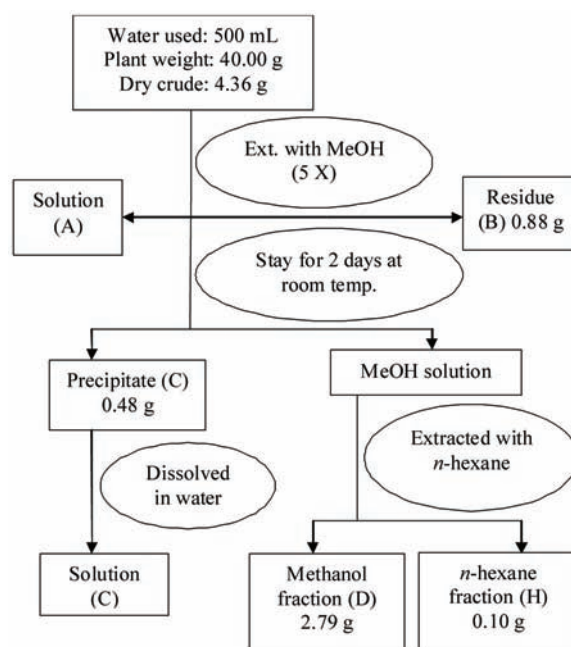
Sclerochloa dura (whole plant) was collected in June–July 2009 from the city of Niš, along the river bank near the Gabrovačka Reka, a creek in Serbia. The plant was identified by Bojan Zlatković from the Department of Biology and Ecology, University of Niš, Serbia. A specimen of the identified plant was deposited in the Herbarium of the Faculty of Science and Mathematics (HMN) of the University of Niš, Serbia (voucher number 6922). Total collected weight of the plant was 250 g. The plant material was dried for 10 days in the dark, with proper ventilation and at room temperature. The dried plant was kept in a closed plastic bag in the dark at room temperature until extraction.

Reagents and chemicals

Water used for extraction was obtained from Millipore Elix 5 water purification system, *n*-hexane was from VWR (USA) and methanol was from Fisher Scientific (UK). Recombinant human IL-1 β was from Roche (UK). Phosphate-buffered saline solution (PBS) was from Oxoid (UK). Labelled (5,6,8,9,11,12,14,15-³H)-arachidonic acid (specific activity 180–240 Ci mmol⁻¹) and liquid scintillation cocktail Ultima Gold were from NEN Perkin Elmer (USA). Dulbecco's Modified Eagle Medium (DMEM), foetal bovine serum (FBS), fatty acid-free bovine serum albumin (fBSA), gentamicin and L-glutamine were from Sigma–Aldrich (USA).

Extraction procedure

Aerial parts were crushed into small pieces and extracted by refluxing with water for 20 min. To enhance extraction, the water extract was sonicated for 15 minutes before and after the refluxing (VWR ultrasound cleaner). After 12 h, the extract was filtered (blue ribbon filter paper from Schleicher and Schuell) and the volume reduced on a rotavapor (Büchi rotavapor R-200). Dry plant sample was obtained by freeze drying (Labconco freeze drier model FreeZone 2.5) at $-70\text{ }^{\circ}\text{C}$ for 60 h. The crude extract was successively extracted, as shown in Scheme 1.

Scheme 1. Separation of crude extract of *S. dura*.

The crude extracts and the subsequent fractions (C fraction – water soluble, insoluble in methanol, D fraction – methanol extract of crude; water soluble as well) were prepared from *S. dura*. The crude extract was tested for anti-inflammatory and antioxidant activities while the subsequent fractions were tested for anti-inflammatory activity only. The *S. dura* crude extract and related fractions were aliquoted in sterile glass vials and stored protected from light at 4 °C prior to use. The *n*-hexane fraction H was not tested for anti-inflammatory activity due to the harmful effect of *n*-hexane on cells.

Culture and treatment of SW982 cells

The human fibroblast-like synoviocyte cell line SW982 was purchased from ATCC (UK) sub-cultured bi-weekly by routine trypsin detachment and kept in a sub-confluent state. The

cells were maintained in DMEM supplemented with 10 % FBS, 0.1 mg mL⁻¹ gentamicin and 0.3 mg mL⁻¹ L-glutamine in a humidified 10 % CO₂ atmosphere at 37 °C. For AA release, 5×10⁵ cells were seeded per well in a 48-well per plate format. Cells were cultivated until 2 days post-confluency, serum starved and labelled with ³H-AA in serum-free DMEM overnight and processed at day 3 post-confluence to ensure differentiation and synchronization of the cells. The experiments were performed in serum-free DMEM in triplicates of the wells and repeated three times. In all experiments, untreated cells without inducing agents or plant extract were included for unstimulated control; distilled water was included for vehicle control. Following the treatments, the cells were routinely microscopically observed to monitor possible effects on cell morphology, integrity and viability.

Arachidonic acid release assay

The AA release assay determines the amount of AA released from SW982 cells stimulated with IL-1β. AA release corresponds to the activation of PLA₂-enzymes that cleave off the AA in the *sn*-2 position of the phospholipid. In the presence of inhibiting compound(s), the release of AA is reduced, which is taken as evidence that the compound(s) target some level in the arachidonyl cascade, such as the PLA₂ enzymes.⁷⁻⁹

At 2 days post-confluency, the SW982 cells were serum-starved and labelled overnight with ³H-AA (0.4 μCi mL⁻¹) in serum-free DMEM. Prior to the addition of the *S. dura* extract or related fractions, the cells were washed with PBS containing fBSA (2.0 mg mL⁻¹) in order to remove unincorporated radioactivity. The cells were pre-treated with various dilutions of the crude extract and subsequent fractions (0–20 % extract in serum-free DMEM, 1 h pre-incubation) followed by the addition of IL-1β (10 ng mL⁻¹) to mimic an inflammatory situation. After 4 and 24 h of IL-1β stimulation, the supernatants were cleared of detached cells by centrifugation (13000 rpm, 10 min). The cellular release of ³H-AA was determined by liquid scintillation counting in an LS 6500 Multi-Purpose Scintillation Counter, Beckman Coulter, Inc. (USA). The adherent cells were dissolved in 1.0 M NaOH in order to determine by liquid scintillation counting the ³H-AA incorporated in the cells. The results are given as released ³H-AA in the supernatants relative to total ³H-AA incorporated in the cells.

Total phenolic content determination

The total soluble phenolic content of the plant extract was determined with Folin–Ciocalteu reagent using pyrocatechol as a standard.^{35,36} An aliquot of 5.0 mg of the dry plant extract was dissolved in 20.0 mL of distilled water in an Erlenmeyer flask. The solution was diluted to 46.0 mL by the addition of distilled water. Folin–Ciocalteu reagent (1 mL) was added to the solution and mixture was shaken vigorously. After 3 min, 3.0 mL of 2 % sodium carbonate solution was added. The flask was covered with aluminium foil to protect the complex from possible effects of light. Flask was shaken occasionally for 2 h at room temperature. The absorbance was measured at 760 nm^{37,38} using a UV mini-1240 – Shimadzu (Tokyo, Japan) spectrophotometer. A standard curve was plotted using pyrocatechol as a standard and the total soluble phenolic contents in the extracts were expressed as μg pyrocatechol equivalent, according to the following equation:

$$Y = 0.0533X + 0.0994 \quad (1)$$

where Y is the absorbance and X the concentration.

Total flavonoid content determination

Dry extract (20.0 mg) was dissolved in 1.0 mL of 80 % ethanol. An aliquot of 0.1 mL was taken and diluted to 1.0 mL, giving a concentration of 2.0 mg mL⁻¹. An aliquot of 0.5 mL

(1.0 mg) was taken and added to a test tube containing 4.3 mL of 80 % ethanol, 0.1 mL of 1 M potassium acetate and 0.1 mL of 10 % aluminium nitrate. The mixture was incubated at room temperature for 40 min. The absorbance was measured at 415 nm using a UV mini-1240 – Shimadzu (Tokyo, Japan) spectrophotometer. The total flavonoid content in the plant extract was expressed as μg quercetin equivalents³⁹⁻⁴¹ using a standard quercetin graph and according to the following equation:

$$Y = 0.0494X - 0.0026 \quad (2)$$

where Y is the absorbance and X the concentration.

Antioxidant activity determination

The antioxidant activity of the extract was determined by the phosphomolybdenum method using α -tocopherol as a standard.^{42,43} One mg of the extract was combined with 2.0 mL of the reagent (0.6 M sulphuric acid, 28.0 mM sodium phosphate and 4.0 mM ammonium molybdate). A blank solution was prepared by mixing 2.0 mL of the reagent solution with the appropriate volume of the same solvent used to dissolve the sample. The tubes were capped and incubated in water bath at 95 °C for 90 min. The sample and blank were left 30 min to cool down to room temperature. The absorbance of the sample was measured against blank solution at 695 nm using a UV mini-1240 – Shimadzu (Tokyo, Japan) spectrophotometer. A tocopherol graph was plotted by using α -tocopherol as a standard and the total antioxidant activity of the plant extract was expressed as μg α -tocopherol equivalents according to the following equation:

$$Y = 7.7686X + 1.678 \quad (3)$$

where Y is the absorbance and X the concentration.

Free radical scavenging activity

The ability of the extract to quench the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) determines the free radical scavenging activity of a plant material.⁴⁴⁻⁴⁶ It is usually expressed as the IC_{50} value^{37,47,48} (the extract concentration required to inhibit the activity of DPPH $\cdot$ by 50 %).

Seven different dilutions of the plant extract were made in 100 % ethanol: 3200, 1600, 800, 400, 200, 100 and 50 $\mu\text{g mL}^{-1}$. A volume of 1.0 mL of 0.3 mM solution of DPPH $\cdot$ was mixed with 2.5 mL of each dilution. All the solutions were left at room temperature for 30 min and then the absorption of each was measured at 518 nm using a UV mini-1240 – Shimadzu (Tokyo, Japan) spectrophotometer. A negative control was prepared by mixing 2.5 mL of ethanol with 1.0 mL of DPPH. The percentage inhibition for all dilutions was determined using the following equation:

$$\% \text{ Inhibition} = 100 - 100(A_{\text{sample}} - A_{\text{blank}})/A_{\text{control}} \quad (4)$$

where A_{sample} is the absorbance of each dilution; A_{blank} is the absorbance of the dilutions without added DPPH $\cdot$ and A_{control} is the absorbance of the solution of DPPH in ethanol.

The graph between probit of inhibition (a unit of measurement of statistical probability based on deviations from the mean of a normal distribution) vs. the log of concentration was plotted and the IC_{50} value of the plant extract was calculated from the graph.

Statistical analysis

All values are expressed as mean $\pm$ SD. The cellular bioactivity data were analysed by the Student's t -test and results were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Physical properties of the crude extract and subsequent fractions

As the traditional use of *S. dura* is reported to be drinking it as a tea, the dried plant was crushed into small pieces and extracted by refluxing with water. From 40.0 g of the dry aerial plant parts, a yield of 4.36 g (10.9 %) of a dark brown coloured crude extract was obtained, whereas 0.48 g (11.0 % relative to dry crude) of yellow coloured water soluble fraction C, 2.79 g (64.0 % relative to dry crude) of dark brown coloured fraction D soluble in both water and methanol and 0.10 g (2.3 % relative to crude) of a transparent hexane-soluble fraction H. In summary, about 80 % of the dried plant extract was extractable by water, methanol and hexane, whereas 20.2 % of the fraction appeared as a black, insoluble residue. The physical properties of the crude extract and the fractions obtained from it are given in Table I.

TABLE I. Colour, yield and solubility of crude extract and subsequent fractions

Fraction	Colour	Yield, %	Soluble in
Crude	Brown	10.9	Water
C-Fraction	Yellow	11.0 ^a	Water
D-Fraction	Brown	64.0 ^a	Water and methanol
H-Fraction	Transparent	2.3 ^a	Hexane
Residue	Black	20.2 ^a	Insoluble

^aRelative to dry crude*The crude extract of S. dura inhibits the release of arachidonic acid*

The *S. dura* extract is traditionally used to alleviate menstrual cramping, excessive bleeding and pain, processes known to involve eicosanoids such as PGE₂,¹³ also recognized as a pro-inflammatory mediator.¹ By use of the AA-release assay and the SW982 cell model system, the effect of the extract on the availability of AA, the rate-limiting precursor for PGE₂ synthesis, was investigated. As shown in Fig. 1, the crude extract of *S. dura* inhibits IL-1 β induced AA release in a dose-dependent manner, with an observed $\approx$ 50 % inhibition at 1.5 mg mL⁻¹ after both 4 and 24 h of stimulation. Hence, the *S. dura* extract may contain anti-convulsive and/or anti-inflammatory compounds that interfere with the arachidonic acid cascade.

The methanolic fraction of S. dura crude extract efficiently inhibits AA-release

Having shown that the crude *S. dura* extract inhibited AA-release, the crude extract was further fractionated into a water-soluble fraction C, a hexane-soluble fraction H and a methanol-soluble fraction D (Scheme 1) in an attempt to identify the active compound(s) in *S. dura*. Both the C and D fraction were tested for their bioactivity in the AA-release assay and they inhibited IL-1 β induced AA-release in a dose-dependent manner, but with different efficacies. The inhibition obs-

erved for fraction D, when the treatments with 0.22, 0.11 and 0.06 mg mL⁻¹ resulted in a 97, 91 and 63 % inhibition of AA-release, respectively, was much higher than that observed for fraction C (Fig. 2A and B). Moreover, compared to the inhibition obtained with the crude extract, fraction D was far more efficient as the IC_{50} value determined for fraction D was in the range of ng mL⁻¹, not mg mL⁻¹ as observed for the crude extract. Fraction H was not tested due to the harmful effect of *n*-hexane to cells. This indicated that most of the bioactive compound(s) responsible for the inhibition of AA-release were located in the methanol-soluble fraction of the crude extract.

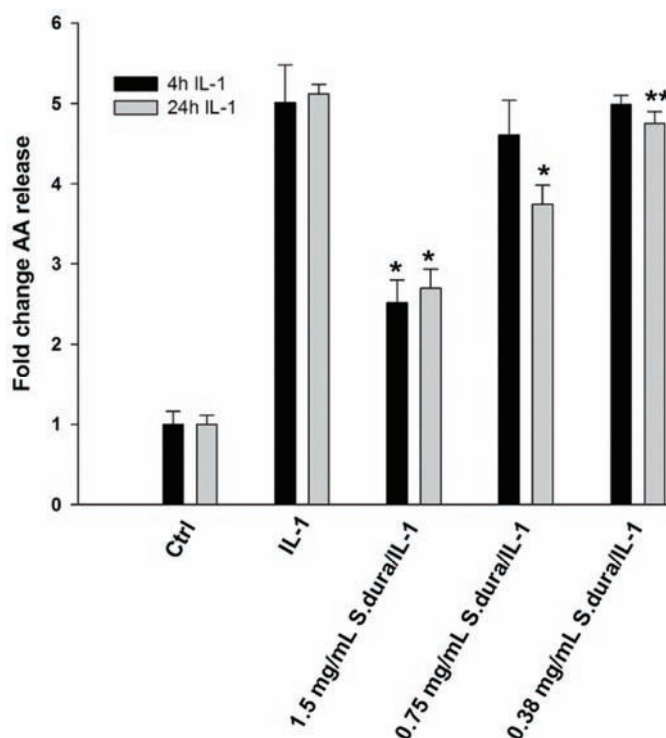


Fig. 1. Crude extract of *S. dura* inhibits IL-1 (10 ng mL⁻¹) induced release of arachidonic acid in a time- and dose-dependent manner in SW982 cells. * $p < 0.001$, ** $p < 0.02$ by Student's *t*-test (means $\pm$ SD of three experiments each performed in triplicate).

Antioxidant activity, phenolic content, flavonoid content and free radical scavenging activity of S. dura crude extract

After demonstrating that the crude extract of *S. dura* was effective in inhibiting AA-release, the antioxidant capacity of the crude extract was determined because a good anti-inflammatory activity often accompanies a good antioxidant activity.^{14,15} The determination of the antioxidant capacity included the quantifi-

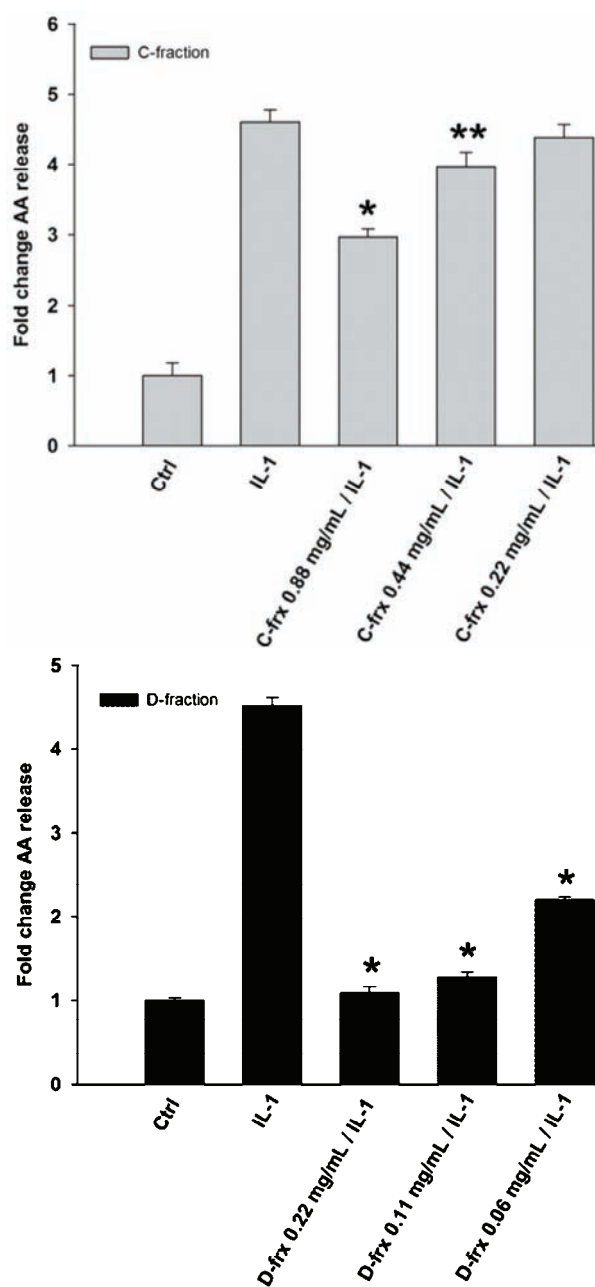


Fig. 2. Different fractions of the *S. dura* crude extract inhibit IL-1 (10 ng mL^{-1} , 24 h) induced AA-release in a dose-dependent manner, but with varying efficacy: a) the water soluble fraction (C) inhibits AA-release at high concentrations and b) the methanol soluble fraction (D) inhibits AA-release more efficiently at lower concentrations. * $p < 0.001$, ** $p < 0.02$ by the students' *t*-test (means $\pm$ SD of three experiments each performed in triplicate).

cation of the antioxidant activity, total phenolic content and the total flavonoid content by the linear regression method and an estimation of the free radical scavenging ability by the DPPH method. The antioxidant activity of the crude

extract of *S. dura* was 70.11 µg equivalents of α -tocopherol, the total soluble phenolic content was 34.78 µg pyrocatechol equivalents and the total soluble flavonoid content was 22.80 µg quercetin equivalents per milligram of the plant extract (Table II). The free radical scavenging activities of several concentrations of *S. dura* extract were determined. The experiment was repeated under same conditions to determine the free radical scavenging activity of ascorbic acid, which was used as a standard. The IC_{50} values for the plant extract and ascorbic acid were determined by calculating the inhibition values for all used concentrations, taking the probit of all inhibition values and plotting them against the log of the respective concentrations. The IC_{50} value for the plant extract was 846.64 vs. 11.77 µg mL⁻¹ for ascorbic acid. The results showed that radical scavenging activities of both ascorbic acid and the plant extract were concentration dependent.

TABLE II. Total antioxidant activity, total soluble phenolic content and total soluble flavonoid content in the extract of *S. dura* compared to *D. falcata* and *W. tomentosa*

Plant	Total content of soluble phenolics, µg pyrocatechol equivalent mg ⁻¹	Total content of soluble flavonoids, µg quercetin equivalent mg ⁻¹	Total antioxidant activity, µg α -tocopherol equivalent mg ⁻¹
<i>S. dura</i>	34.78±0.38	22.80±0.16	70.11±0.66
<i>W. tomentosa</i>	7.20±0.88	16.90±1.00	4.20±0.03
<i>D. falcata</i>	38.66±1.86	21.59±1.09	—

In a quest to determine the antioxidant potency of *S. dura*, the obtained results were compared with the results reported in literature for the aerial parts of *Dendrophthoe falcata* (Loranthaceae) and the leaf extract of *Wrightia tomentosa* (Apocynaceae) (Table II). Both of these plants chosen for comparison have traditional use for the treatment of menstrual disorders,^{32–34} i.e., a similar ethnopharmacology to *S. dura*.

The comparison showed that *S. dura* had a 4.8 times higher total soluble phenolic content, a 1.3 times higher quercetin equivalent flavonoid content and a 17 times higher total antioxidant activity than *W. tomentosa*.⁴² A comparison of crude extracts of both plants relative to the DPPH scavenging ability of ascorbic acid showed that the extract of *W. tomentosa* was 1.6 times more potent than the extract of *S. dura*.⁴² On the other hand, there was not much difference in the total soluble phenolics and flavonoids content of *S. dura* and *D. falcata*.³⁴ The limitation in comparing the antioxidant activity and free radical scavenging activity of *S. dura* and *D. falcata* was the non-availability of published results of the α -tocopherol equivalent antioxidant activity and DPPH scavenging ability compared to ascorbic acid of *D. falcata*. In summary, the antioxidant capacity of

S. dura is similar to that of *D. falcata* and far better than that of *W. tomentosa* except in terms of its free radical scavenging ability.

CONCLUSIONS

The results from the cellular testing of *S. dura* (crude extract and sub-fractions) in the AA-release assay suggested that the reported pain- and discomfort-relieving effects of the plant may be explained by the strong inhibitory effects on the arachidonic acid cascade. By reducing the availability of AA, the synthesis of eicosanoids may be reduced accordingly. Moreover, the total soluble phenolics and flavonoids contents of *S. dura* were similar to those of *D. falcata* but higher than those of *W. tomentosa*. Although *S. dura* is less effective in the DPPH assay, it has higher antioxidant properties compared to *W. tomentosa*.

The presence of flavonoid compounds, known for their various pharmacological activities, a considerably high antioxidant property and the fact that *S. dura* may exert anti-convulsive, anti-inflammatory and pain-relieving properties, gives reasons to believe that the use of the plant in traditional medicine has a solid chemical background. Identification of the active compound(s) is the next step in the study of *S. dura*, which could, potentially, reveal new drug candidates for various indications.

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ИЗВОД

АНТИИНФЛАМАТОРНА И АНТИОКСИДАТИВНА АКТИВНОСТ БИЉКЕ *Sclerochloa dura* (POACEAE)

SYED MAJID BUKHARI¹, ASTRID JULLUMSTRØ FEUERHERM², FAYSSAL BOULFRAD³, БОЈАН ЗЛАТКОВИЋ⁴,
BERIT JOHANSEN² и НЕБОЈША СИМИЋ¹

¹Department of Chemistry, Norwegian University of Science and Technology, 7491 Trondheim, Norway,

²Department of Biology, Norwegian University of Science and Technology, 7491 Trondheim, Norway,

³UFR de Pharmacie, Grenoble, France и ⁴Природно-математички факултет, Ниш,

Универзитет у Нишу, 18000 Ниш

Биљка *Sclerochloa dura* се традиционално користи у југоисточној Србији за олакшавање менструалних тегоба, као што су бол и претерано крварење. Према искуству корисница, убрзо по узимању биљке, смањује се крварење и бол. Циљ овог испитивања је да се утврди инхибиторни ефекат биљке на активност каскаде коју започиње арахидонска киселина (AA) и да се спектрофотометријски одреди антиоксидативна активност. Тест ослобађања AA изведен је коришћењем хумане ћелијске линије синовиоцита SW982, која је слична фибробластима, а мерена је активност фосфолипазе A2 (PLA₂). Сирови екстракт и изоловане фракције *S. dura* инхибирају отпуштање AA које иницира интерлеукин 1, на временски и дозно зависан начин. IC₅₀ сировог екстракта је 1,5 mg mL⁻¹ након 4 h, односно 24 h стимулације. Третирање ћелија метанолним екстрактом концентрација 0,22, 0,11 и 0,06 mg mL⁻¹ довело је до инхибиције отпуштања AA од 97, 91, односно 63 %. Један милиграм сировог екстракта је садржао фенолна једињења која

би одговарала 34,78 μg пирокатехола и флавоноида који би били еквивалентни 22,80 μg кверцетина. Антиоксидативна активност је била еквивалентна активности 70,11 μg α -токоферола. Јак инхибиторни ефекат екстракта *S. dura* на активацију АА каскаде би могао објаснити смањење бола и других неугодних осећаја.

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