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Aqueous Extract Of Tutsan (*Hypericum Androsaemum L.*) Red Berries Exhibits Antioxidant Activity And Effects Akin To Those Of An Antidepressant

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Abstract:
Hypericum androsaemum L., sometimes referred to as "tutsan" or "shrubby St. John's Wort," is a plant in the Hypericum genus that grows naturally in the Mediterranean region. It is widely grown as an ornamental plant because of the striking color variation in its young, berry-like capsules, which turn shiny black as they ripen from red. In traditional medicine from Portugal and Spain, tutsan has also been used to treat depression. We evaluated the potential benefits of H. androsaemum red berry water extract (WE). After H. androsaemum red berries were decocted, WE was produced, and its ten bioactive component content was ascertained using HPLC-DAD analysis. By boosting endogenous antioxidant defenses and restoring normal behavior, the maximum dosage of WE was found to effectively alleviate the symptoms of depression overall and lower oxidative stress levels.

Keywords: Tutsan, Red berries, Antidepressant, Hypericum androsaemum L., etc.

Introduction:
Stroke is one of the most significant cerebrovascular illnesses, with high global death and disability rates [1]. There are two types of stroke: ischemic and hemorrhagic. The latter results in brain damage and infarction, and cerebral blood flow is compromised by low blood sugar and oxygen levels [2]. Reactive oxygen species (ROS), which lead to neuronal malfunction and death, are produced as a result, changing antioxidant defenses [3]. Major depressive disorder (MDD) is characterised by severe biological molecular damage that leads to neurotoxicity and neurodegeneration [5], and this oxidative stress plays a role in its development [4]. Therefore, common symptoms that follow an acute ischemic stroke include anxiety, depression, psychosis, apathy, dementia, and chronic fatigue syndrome. [6]. Depression is one of these, affecting stroke patients frequently and being a major neuropsychiatric condition [7]. Poor recovery outcomes, social issues, isolation, cognitive impairment, sleeplessness, and an increased risk of death are common symptoms of post-strokedepression (PSD) [6, 8, 9].

The primary therapeutic approach for treating PSD is the use of antidepressant medications; however, these frequently have substantial side effects that can aggravate morbidity and even cause therapy to be stopped [10]. PSD clinical symptoms bear resemblance to those observed in major depressive disorder cases. Thus, oxidative stress is crucial for both major depressive disorder and associated psychiatric diseases, as well as chronic inflammation [11, 12]. We have previously postulated that oxidative stress is a significant factor in stroke-induced damage as well as serious depressive disorders [6, 13]. Our search for herbal remedies with antioxidant and antidepressant qualities that could be clinically useful in the treatment of depression following a stroke led us to concentrate on the Hypericum genus (Hypericaceae family). A variety of conventional herbal medications and dietary supplements contain extracts from the aerial portions of Hypericum perforatum L., which are commonly used as a depression treatment [14]. Known as "tutsan" or "shrubby St. John's Wort," Hypericum androsaemum (sect. Androsaemum) has been little research done on its pharmacological properties to date. The Mediterranean shrub Tutsan grows in forests where oak and chestnut trees predominate. H. androsaemum is referred to as "iperico arbustivo," "ruta sana," "erba di San Giovanni arbustiva," and other local terms in Italy [15]. H. androsaemum has fleshy, berry-like capsules that turn black as they ripen, in contrast to most other Hypericum species, which have dry capsules as fruit [16, 17]. Another characteristic that sets Tutsan plants apart is their lack of black leaf nodules, which are secretory structures where naphtodianthrones are formed [18]. Consequently, hypericin and its compounds are absent from the resultant plant extracts [16]. In the Mediterranean region, Hypericum androsaemum is one of the most widely used herbal remedies in the genus, behind St. John's Wort. While its aerial sections are infused and used to treat anxiety and depression in Spain [22], it is known as "Hipericao do Geres" in Portugal and is used as a hepatoprotective, diuretic, and antidepressant [19–21]. While methanolic extracts from H. androsaemum aerial parts have been shown to have depressive qualities in vivo [21], the fruits' potential for medicinal application has not been investigated.In a previous study, Based on our earlier research, we postulated that the antioxidant capacity of berries like H. androsaemum could be beneficial in managing post-stroke depression symptoms. Thus, we tested water extracts from H. androsaemum red berries for possible antidepressant and antioxidant benefits in post-stroke depressive-like effects in mice brains.

Material and MethodPlant material

In July, red berries were harvested from Hypericum androsaemum L. This stage of the fruit provides the maximumconcentrations of bioactive components, such as phenolic acids and flavonoids, according to earlier studies [16, 23].

Fig. 1. Rosemary with hypericum spp. berry-like red capsules that were examined in the researchReagents and standards

The following analytical reference samples were acquired from Sigma-Aldrich: hyperoside, quercitrin, rutin, 3, 5-di-O-caffeoylquinic acid, chlorogenic acid, neochlorogenic acid, shikimic acid, epicatechin, and gallic acid. Vials were stored at 4 °C in the dark after each analyte was dissolved in methanol to create stock standard solutions with a concentration of 1000 mg/1000 mL. Every day, new standard solutions were made. Sigma-Aldrich supplied HPLC-grade acetonitrile and methanol, while J.T. Baker B.V. supplied HPLC-grade formic acid. Deionized water ≥ 18 MΩ/cm resistivity, purified using a Milli-Q system was utilized for the preparation of samples and chromatographic analyses. Furthermore, Supelco supplied 0.45-μm PTFE filters, which were used to filter all solvents and solutions prior to usage.

Preparations of water extracts

After macerating 50 g of crushed red fruits in 300 mL of boiling water for 30 minutes, water extract (WE) was produced (producing 17.09 %, w/w dry weight). Using a Rota vapor, the extract was concentrated, lyophilized, and stored at 4 °C in the freezer until needed. Prior to chromatographic analysis, 10 mg of extract were filtered out of a sample solution made upof 1 mL of water for HPLC analysis.

Biochemical investigationLipid peroxidation

Thiobarbituric acid-reactive substance (TBARS), a marker for lipid peroxidation, was quantified using a method developedby our group [29, 30]. Samples of homogenised brain tissue with 1 mg of protein were mixed with about 1 mL of 20% trichloroacetic acid and 2 mL of 0.67% thiobarbituric acid. After an hour of incubation at 100 °C, the mixture was cooled and the precipitate was removed by centrifuging the samples. The absorbance of the reagents, excluding brain homogenate, was measured at λ = 532 nm in reference to a blank in order to provide a control for the reaction mixtures.

Superoxide dismutase

We used the Misra and Fridovich [31] procedure to measure the activity of superoxide dismutase (SOD). In short, 0.2 mL of freshly prepared hydroxylamine hydrochloride (0.1 mM), 0.4 mL of nitroblue tetrazolium (25 M), and 1 mL of sodium carbonate (50 mM) were combined to create a combination. Next, 0.1 mL of the clear supernatant of the homogenate (1:10,% w/v) was added. Variations in absorbance were measured at λ = 560 nm.

Reduced glutathione levels

Glutathione (GSH) level drop was examined using Ellman's approach [32]. The protein was precipitated using trichloroacetic acid (5%) after homogenates (720 μL) were diluted twice. The supernatant was collected following centrifugation (12,000 g, 5 min), and Ellman's reagent (5, 5-dithiobis 2-nitrobenzoic acid solution) was then added. The absorbance of each sample was then determined at λ = 417 nm. Next, a standard curve was created using reduced glutathione solution concentrations that were known.

Catalase activity

In this work, a slightly modified version of Sinha's [33][29] technique was used to quantify catalase activity.In summary, 2. 1 mL of 7.5 mM hydrogen peroxide was combined with a homogenate volume containing 5 μg of protein.The absorbance of the reaction mixture was measured at λ = 240 nm after 10 minutes.

Statistical analysis

IBM SPSS 21.0 for Windows, a statistical application for the social sciences, was used for statistical analysis. The results were shown as means ± standard deviation (SD), and statistical significance was established at $p < 0.05$ using a oneway variance analysis. The Bonferroni post-hoc test was used to find any significant differences between the groups.

Result and Discussion

HPLC analysis of the water extract

For this study, ten bioactive marker molecules were evaluated in the red berry H. androsaemum water extract (WE) (TableThe target analytes represented 180.6 g/kg of WE, of which the three main components were catechin (5.8 g/kg), chlorogenic acid (56.9 g/kg), and shikimic acid (110.0 g/kg) (Fig. 2). Hyperoside, another common secondary metabolite, was discovered in flavonol glycoside at a concentration of 2.7 g/kg.

Table 1: Marker component content in the Hypericum androsaemum red berry water extract

Compound	Content (g/kg dw)
Shikimic acid	111.1 ± 3.0
Gallic acid	0.6 ± 0.03
Neochlorogenic acid	1.1 ± 0.02
Chlorogenic acid	57.8 ± 1.3
3,5-di-O-caffeoylquinic acid	n.d.
Catechin	5.9 ± 0.4
Epicatechin	2.1 ± 0.09
Rutin	1.7 ± 0.2
Hyperoside	2.8 ± 0.09
Quercitrin	n.d.
Total	181.7 ± 1.6

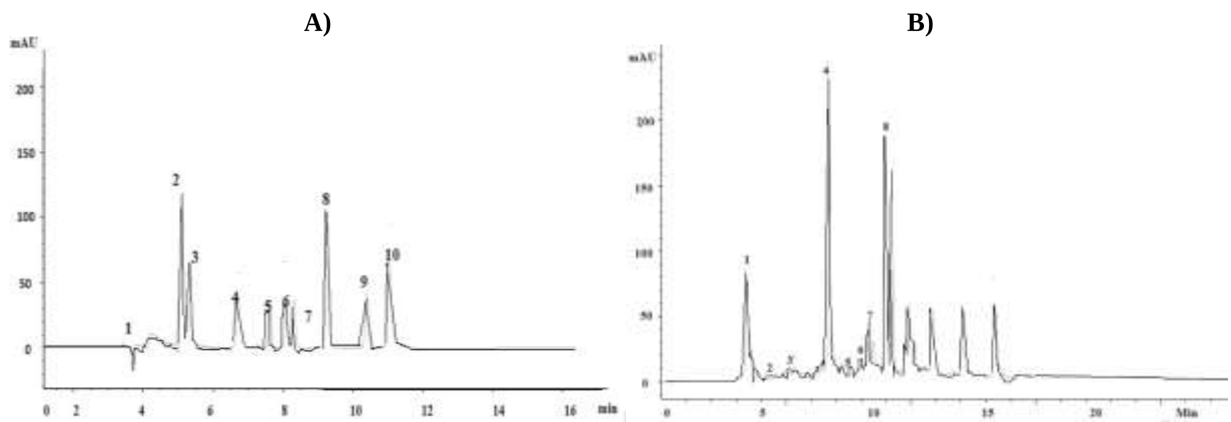

Fig. 2: Chromatographs using HPLC-DAD. They are given as follows for the purpose of clarity: A) reference mixture solution containing the ten analytes under survey, B) aqueous extract of H. androsaemum. The measurements are taken at 256 nm. List of substances 1, 2, gallic acid, 3, 5-O-caffeoylquinic acid, 4 3-O-caffeoylquinic acid, 5 epicatechin, 6 catechin, 7 rutin, 8 hyperoside, 9 quercitrin, and 10 3,5-di-O-caffeoylquinic acid.

After administering WE at both tested doses, the antioxidant properties of WE were assessed. The induction of stroke led toan increase in TBARS levels and a significant ($p < 0.01$) decrease in the activity of endogenous antioxidant defenses, including SOD, GSH, and Cat. (Fig. 6A–D). The extracts exhibited impressive antioxidant activity, despite their inability toreturn these endogenous antioxidant molecules to normal physiological quantities. At the higher concentration tested (30 mg/kg), WE was determined to be the most effective treatment (Fig. 6A–D).

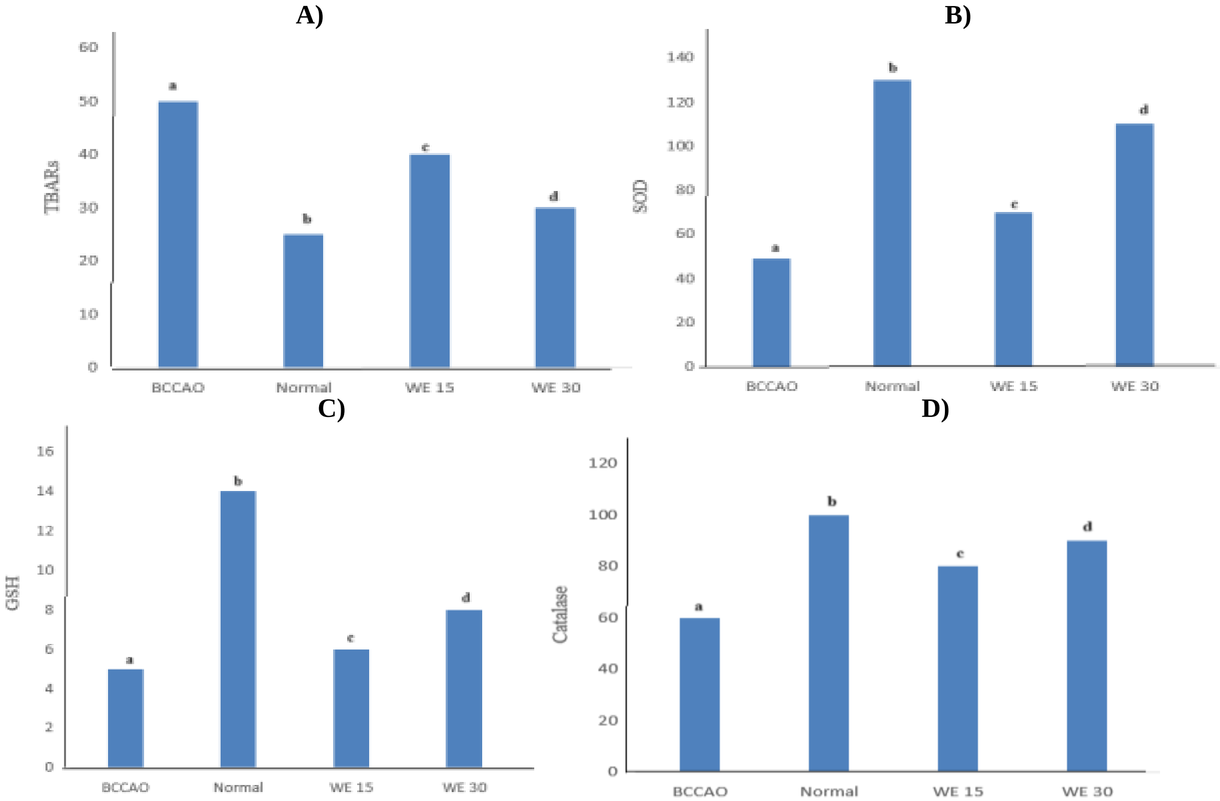

Fig. The impact of red berry extracts (WE) from Hypericum and Rosaemum on oxidative stress. Intraperitoneal WE administration's effects on the following parameters in mouse brain tissue: glutathione (GSH) levels, expressed as µg/mg protein (C), catalase activity, expressed as U/mg protein (D), superoxide dismutase (SOD) activity, expressed as nmol MDA equivalent/g tissues (A), and Glutathione (TBARS) levels, expressed as µg/mg protein (B). The data are presented asmeans ± SD ($n = 7$); significant differences ($p < 0.05$) between the two groups are indicated by different letters.

The relationships between the antioxidant activity and the antidepressant-like effects found in behavioral data were investigated using Pearson's linear correlation Antioxidant enzymes actually demonstrated a high positive link with both movement metrics, but TBARS exhibited a substantial negative relationship (Table 2).

Conclusion

The polar extract from the fruit of *H. androsaemum*, a well-known Mediterranean medicinal plant, has been shown in this work to have health-promoting properties. We expect that more research will uncover this portion of the *H. androsaemum's* potential, which may subsequently be used to make nutraceuticals and functional foods that protect against oxidative stress and neurodegenerative illnesses.

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