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ASSESSMENT OF HEMOSTATIC ACTIVITY OF THE AQUEOUS EXTRACT OF LEAVES OF MARRUBIUM VULGARE L, A MEDITERRANEAN LAMIACEAE ALGERIA

Nabil Ghedadba
Laboratory of Materials Chemistry and Living: Activity and Reactivity. Department of natural science and life, Faculty of Science, El- Hadj- Lakhdar University 05000. Batna, Algeria
viamessi@gmail.com

Leila Hambaba
Laboratory of Materials Chemistry and Living: Activity and Reactivity. Department of natural science and life, Faculty of Science, El- Hadj- Lakhdar University. 05000. Batna, Algeria
viamessi@gmail.com

Nassima Fercha
Laboratory of Materials Chemistry and Living: Activity and Reactivity. Department of natural science and life, Faculty of Science, El- Hadj- Lakhdar University. 05000. Batna, Algeria,
viamessi@gmail.com

Bousselsela Houas
Laboratory of Materials Chemistry and Living: Activity and Reactivity. Department of natural science and life, Faculty of Science, El- Hadj- Lakhdar University. 05000. Batna, Algeria,
viamessi@gmail.com

Samira Abdessemed
Laboratory of Materials Chemistry and Living: Activity and Reactivity. Department of natural science and life, Faculty of medicin
miramed_2006@yahoo.fr

Sidi Mohamed Ould Mokhtar
Unité de Biodiversité et Valorisation des Ressources Végétales (BVRV) – Faculté des Sciences et Techniques (FST)- Université des Sciences, de Technologie et de Médecine (USTM) Nouakchott, Mauritanie
issawi41@gmail.com

Abstract

The overall objective of this study was to evaluate in vitro the hemostatic activity of secondary metabolites (polyphones, flavonoïds and tannins) of Marrubium vulgare leaves, aromatic plant widely used in traditional medicine for the treatment of asthma, cough, diabetes (by its effect on the pancreas to secrete insulin), heart disease, fever has a high efficiency as against inflammation. Qualitative analysis of the aqueous extract (AQE) by thin layer chromatography revealed the presence of quercetin, kaempferol and rutin. Quantification of total phenols by Folin Ciocalteu method and flavonoïds by AlCl3 method gave high values with AQE: 175 ± 0.80 mg GAE per 100g of the dry matter, 23.86 ± 0.36 mg QE per 100g of dry matter. Moreover, the assay of condensed tannins by the vanillin method showed that AQE contains the highest value: 16.55 ± 0.03 mg E-Catechin per 100g of dry matter. Assessment of hemostatic activity by the plasma recalcification method (time of Howell) has allowed us to discover the surprising dose dependent anticoagulant effect of AQE lyophilized from leaves of M. vulgare. A positive linear correlation between the two parameters studied: the content of condensed tannins and hemostatic activity (r = 0.96) were used to highlight a possible role of these compounds that are potent vasoconstrictor activity in hemostatic. From these results we can see that Marrubium vulgre could be used for the treatment of health.

Keywords

Marrubium Vulgare L, Aqueous Extract, Phenolic Compounds Dosing, Hemostatic Activity, Condensed Tannins

1. Introduction

Analysis of the blood such as blood coagulation analysis to prevent bleeding or avoid suffering from thrombosis, that is to say producing in vivo clots in the heart or vascular cavities

we conduct search of bioactive molecules natural present in a plant capable of coagulating blood tested in the assessment of haemostatic activity. Marrubium vulgare (Lamiaceae), mainly rich in phenol compounds, is widely used in traditional medicine to cure certain diseases. M. vulgare has hypoglycemic and lipid-lowering effects (Elberry et al., 2011), vasorelaxant and antihypertensive (El Bardi et al., 2004), anti-cholinesterase against the acetyl cholinesterase (Orhan & al., 2010), antioxidant and anti-inflammatory (Stulzer et al., 2006).

This potential of biological activities is due to the richness of M. vulgare with active ingredients: diterpenes like marrubiin and marrubenol (Stulzer et al, 2006), flavonoids as ladanein, the apigenol and the quercetol (Elberry et al., 2011), essential oils such as eugenol, β- caryophyllene (Orhan et al., 2010), phenolic acids such as gallic acid and tannins. The principal objectives of the study were as follows.

- To determine the content of the extracts of M. vulgare secondary metabolites especially in condensed tannins.
- To evaluate in vitro the hemostatic activity extracts of leaves of this plant by the recalcification of decalcified plasma method.

For this reason, the research was to study polyphenols content: quantification of total phenols by Folin Ciocalteu method and flavonoids by AlCl3 method gave high values with AQE: 175 ± 0.80 mg GAE per 100g of the dry matter, 23.86 ± 0.36 mg QE per 100g of dry matter. Moreover, the assay of condensed tannins by the vanillin method showed that AQE contains the highest value: 16.55 ± 0.03 mg E-Catechin per 100g of dry matter. In the present article, the authors present the value of these secondary metabolites (Wojdylo et al, 2007). The distribution of secondary metabolites can be varied during the development of the plant under certain conditions such as salinity, drought and sun exposure that act on the biosynthesis of secondary metabolites such as polyphenols. According Wojdylo et al. (2007), the content of phenolic compounds varied also depending on the method of extraction.

Table 1.1: *Flavonoïds Content, Polyphones and Condensed Tannins in the Extracts of M. vulgare*

Flavonoïds dosing	Polyphones content	Condensed Tannins
175 ± 0.80	23.86 ± 0.36	16.55 ± 0.03
mg GAE per 100g of the dry matter	mg QE per 100g of dry matter	mg E-Catechin per 100g of dry matter
Wojdylo et al., 2007	Ghedadba et al., 2014	Ghedadba et al., 2014

1.2 Thin Layer Chromatography (TLC)

The thin-layer chromatography after visualization by UV at 254-366 nm and Godin reagent has shown several spots in each extract. After the comparison with the available standards migrated in the system CHCl3 / Me OH / H2O (65: 35: 5) was revealed the likely presence of gallic acid (Rf = 0.82) which was identified previously in our work (Ghedadba et al., 2014), quercetin (Rf = 0.66), rutin (Rf = 0.51), kaempferol 3-O-glucoside (Rf = 0.62) in the EMe OH. These flavonoïds have been identified and purified from various plants. For example, Rigano & al (2009) have identified and purified 11 flavonoïds from the methanol extract of the plant Marrubium glob sum with kaempferol-3-O-glycoside and quercetin. In addition, two new metabolites were revealed for the first time: trans-Cinnamic acid: C9H8O2 (Rf = 0.59) and 4-hydroxybenzoic acid: C7H6O3 (Rf = 0.54). The spots chromatograms petroleum ether extracts (Rf 0.18; 0.34; 0.47; 0.71), dichloromethane (Rf 0.25; 0.47; 0.72; 0.69) is color purple after revelation Godin reagent: these are probably sterols and triterpenes.

After the exposure with a methanol solution of DPPH at 2 mg / ml (indicated by the anti-radical activity), the dichloromethane extracts, butanol, methanol and aqueous gave yellow spots, indicating that the antioxidant compounds included by each extract have the ability to reduce DPPH radical.

Figure 1: *TLC of the Extracts Leaves of M. Vulgare*

1.3 Hemostatic Activity

Marrubium vulgare precipitate blood proteins involve the activity of this plant as an astringent. It is important to stress here that this astringent activity promotes vasoconstriction, which is an important parameter in hemostasis. This vasoconstriction is caused by the presence of tannins in this plant. These observations are similar to Aouissa (2002) who found that the astringent property leaves Mangifera indica was linked to their tannins.

Indeed, tannins have a hemostatic and vasoconstrictive effect on small vessels, and their use against varicose veins and hemorrhoids. Also tannins used orally, are vasoprotective; they limit fluid loss and promote tissue regeneration in case of injury or superficial burn (Bruneton, 1999).

According to our results the content of condensed tannins gave a higher value and very important 16.55 ± 0.03 mg CE per 100g of dry matter, it indicates the richness of aqueous extract with tannins. Therefore, a positive linear correlation between the two parameters studied: condensed tannin content and hemostatic activity (r = 0.96) allowed highlighting a probable role of these compounds that are vaso-constrictors powerful in the hemostatic activity. This may be due to the phenol hydroxyl groups of tannin as the catechin capable of reacting with strong hydrogen bonds with the atoms of the peptide binding protein inhibiting thrombin for example, a proteolysis enzyme that converts fibrinogen a soluble molecule in a molecule insoluble fibrin.

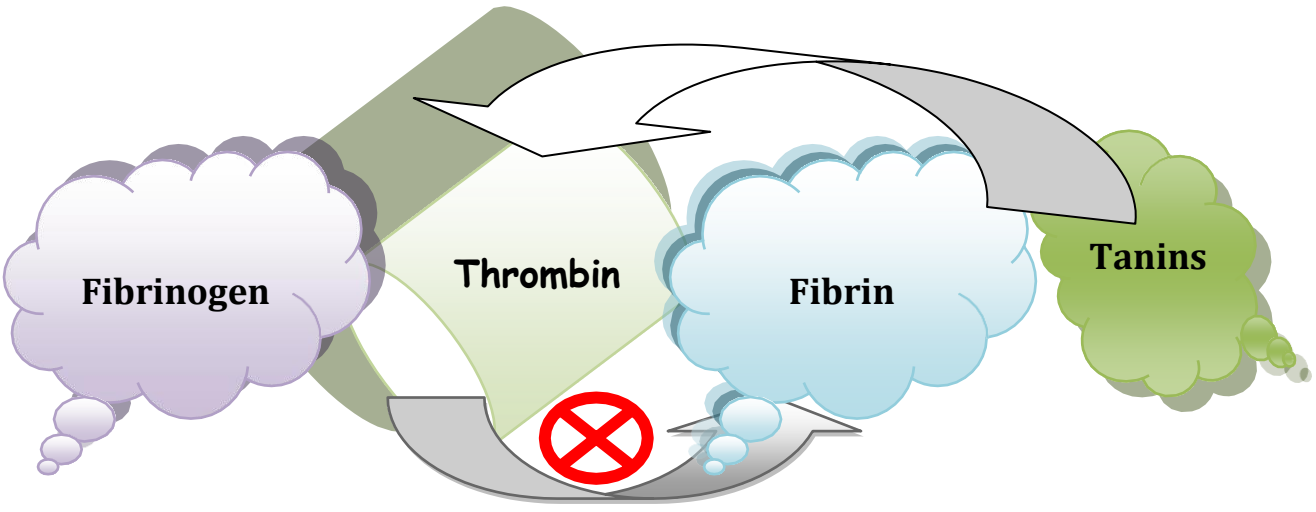

Figure 2: *Hemostatic Activity of Condensed Tannins of M. Vulgares*

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