PHYTOCHEMICAL SCREENING OF *CERATONIA SILIQUA* BARK AND LEAF

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Abstract

Ceratonia siliqua L. is a Mediterranean plant that is widely used in traditional medicine and in food for its fruit and seeds. Leaves and bark are therefore by-products of cultivation but are not valued. The objectives of this study is to compare the chemical components of the leaf and bark and to determine the different chemical classes present in these two parts. This is a descriptive comparative study by a tri-phytochemical screening of the leaf and bark of Ceratonia siliqua. The extraction is performed by three solvents of increasing polarity for each organ. On the six extracts obtained, characterization reactions of the different chemical groups are carried out. As result, both organs contain sterols, saponosides as well as polyphenols including catechic tannins but only the leaf contains flavonoids characterized in the three extracts of this organ. On the other hand, the reactions of alkaloids and quinones are negative for both organs, it would seem that this plant ensures a certain safety for their food and therapeutic use. The leaf proved very interesting by the presence of flavonoids known to date for their therapeutic virtues. These encouraging results must be supplemented by assays as well as bioassays and clinical trials.

Keywords: *Ceratonia siliqua* L., carob tree, phytochemical screening, flavonoids

1. INTRODUCTION

Ceratonia siliqua L. (carob tree) is a widespread forest tree around the Mediterranean (Biner, 2007). According to Food and Agriculture Organization (FAO) , annual world production is estimated at 310,000 tonnes. Note that Algeria is also one of the producers and exporters of carob thanks to some plants well known worldwide (Naghmouchi, 2009). In the other hand , in Algeria, carob is of great interest in therapy and traditional medicine (Azab,2017). However, few studies are done on leaf and bark.The present work is a contribution to the valorization of the by-products of this species. It aims to identify the main chemical groups present in leaf and bark and to compare results between the two parts.

2. METHODS

This is a descriptive comparative study by tri-phytochemical screening of the leaf and bark of *Ceratonia siliqua* L. It covers a period of eight months from October 2021 to May 2022.

2.1. Harvesting and drying

The plant is harvested in Tlemcen in western Algeria at GPS coordinates: 35°04'06.6"N 1°25'48.6"W. The whole branches harvested are thoroughly washed and sent to the laboratory for identification, debarking and fragmentation. The bark and leaf are dried away from light and moisture at room temperature. After that, they are finely ground with mortar and/or automatic grinder.

2.2. Extraction

To carry out the tri-phytochemical study, on the powder of the two organs (leaf and bark), three extractions are carried out according to the protocol developed by Nemlin and Brunel (1995). The raw extracts are obtained by successive extractions with solvents of increasing polarities used in this order: petroleum ether, methanol and distilled water. For petroleum ether extraction, 20 g of powder of each drug is put in contact with 60 ml of petroleum ether. The mixture is homogenized by hand stirring for 10 min. The mixture is then filtered. The resulting filtrate is called ethereal filtrate 1. On the marc, 60 ml of petroleum ether are added, after 10 min of agitation and filtration, the ethereal filtrate 2 is obtained. The same operation made it possible to obtain the ethereal filtrate 3. These 3 filtrates are grouped together and concentrated at 25 ml on a sand bath. This series of operations led to a concentrated solution called etheric extract. After depletion with petroleum ether, the residual marc is dried. The resulting powder is recovered in 60 ml of methanol. 10 min of homogenisation by manual agitation and then filtartion allowed to obtain the methanol filtrate 1. The same operation is repeated to give the methanol filtrate 2. These last two filtrates are combined and concentrated to 25 ml, to the sand bath, to give the methanol extract. To prepare the aqueous extract, 5g of the dry powder of each drug is infused in 50 ml of distilled water, for 15 min. The infused has been filtered to obtain the aqueous extract.

2.3. Reactions of phytochemical screening

On the six extracts obtained, characterization reactions of the different chemical groups are carried out. The different chemical groups are characterized according to the protocol described in the work of Ronchetti and Russo (1971), Wagner (1983), Békro (2007). Polyphenols are characterized by the reaction to ferric chloride: on 2 ml of each extract a drop of alcoholic solution of ferric chloride 2% is added. The appearance of a more or less dark blue-blackish or green coloration indicates the presence of polyphenols. Flavonoids are sought by the reaction to cyanidin: 2 ml of each extract are evaporated and the residue is included in 5 ml of hydrochloric alcohol diluted twice. By adding 2 to 3 shavings of magnesium, there is a release of heat then appartition of a pink orange or purplish color. The research of catechic tannins is carried out by the Stiasny reagent: 5 ml of each extract are evaporated dry, the residue is taken up by 15 ml of Stiasny reagent then heated in a bath-bathThe observation of a precipitate in large flakes characterizes the catechic tannins. For gallic tannins, the previous solution is filtered and the filtrate is recovered and saturated with sodium acetate. The addition of 3 drops of 2% ferric chloride causes an intense blue-black colouration.Alkaloids are characterized by Bouchardat (iodine) and Dragendorff (potassium iodo-bismuthate) reagents. 6 ml of each solution has been evaporated dry. The residue is taken up by 6 ml of alcohol at 60°. The addition of 2 drops of the Dragendorff reagent on the alcoholic solution forms an orange-coloured precipitate. The addition of 2 drops of the Bouchardat reagent on the alcoholic solution forms a precipitate of reddish brown colouring and indicated a positive reaction.To search for saponosides, the foam index is calculated using only the aqueous extract: in a test tube, 10 ml of the total aqueous extract are agitated for 15 seconds then left at rest for 15 min. A persistent foam height, greater than 1 cm indicates the presence of saponosides. Quinonic substances are sought by the Borntreager reagent: 2 ml of each of the three extracts are evaporated dry. The residue is milled in 5 ml of hydrochloric acid diluted to 1/5. The triturât is poured into a test tube and then put in the water bath for 30 min. After cooling, it is extracted by 20 ml of chloroform. 0.5 ml of ammonia diluted twice is added to the chloroformic solution. A red or purple coloration indicates the presence of quinones. Finally, sterols and polyterpenes are sought by the reaction of Liebermann: 5 ml of each of the three extracts are evaporated on a sand bath. The residue is dissolved hot in 1 ml of acetic anhydride, 0,5 ml of concentrated sulphuric acid is added to the triturat. The appearance, at the interphase, of a purple or violet ring, turning blue then green, indicates a positive reaction.

3. RESULTS

The results of the different reactions on the different extracts are expressed in Table 1. Positive reactions to polyphénols in leaf and bark are shown in Figure 1. Positive reaction to flavonoids in leaf is shown in Figure 2.

Table 1

Results of tri-phytochemical screening reactions. +: positive reaction, -: negative reaction

Reaction	**Bark extracts**			**Leaf extracts**		
	etheric	**Methanolic**	**aqueous**	**etheric**	**methanolic**	**Aqueous**
Polyphenols	-	+	+	-	+	+
Flavonoids	-	-	-	+	+	+
Catechic tannins	+/-	+	+	+/-	+	+
Haydrolysable tannins	-	-	-	-	-	-
Saponosids	/	/	+	/	/	+
Alkaloids	-	-	-	-	-	-
Quinonic substances	-	-	-	-	-	-
Sterols and polyterpenes	+	+	+	+	+	-

Figure 1. Positive reaction to polyphenols in leaf and bark.

Figure 2. Positive reaction to flavonoids in leaf.

4. CONCLUSION, DISCUSSION AND RECOMMENDATIONS

The phytochemical study of the different bark extracts of *Ceratonia siliqua* L. shows a relative richness in secondary metabolites. These results are confirmed with studies of El-Hajaji (2011) and Lachkar (2016), such as the presence of polyphenols, catechic tannins, sterols, saponosides and polyterpenes and absence of alkaloids. The analysis also revealed a discrepancy in the absence of flavonoids and the presence of saponosides, this can be explained by the difference of several parameters the environment of the plant, the light, the harvest season, phytochemical (products and techniques used) or biological (genetic heritage). The tri-phytochemical analysis shows that the *Ceratonia siliqua* L. leaf contains polyphenols including flavonoids and catechic tannins, saponosides and sterols and the absence of gallic tannins, alkaloids and quinonic substances. These results are in line with the study carried out by the phytochemical screening of Sassi (2016).

Polyphenols, saponosides, catechic tannins as well as sterols and polyterpenes are present in both organs. In contrast, alkaloids, quinonic substances and gallic tannins are absent. The two organs therefore have a chemical composition which is qualitatively similar.

Ceratonia siliqua L. is one of the most important trees in the Middle East and Mediterranean basin. A qualitative phytochemical screening by reaction has been developed on the leaf and bark parts which aims to characterize chemical substances likely to be used in several fields.The leaf proved very interesting by the presence of flavonoids known to date for their therapeutic virtues. These encouraging results must be supplemented by assays as well as bioassays and clinical trials.

5. RESSOURCES

Azab, A. (2017). CAROB (Ceratonia siliqua) : HEALTH, MEDICINE, CHEMISTRY. European Chemical Bulletin, 6(10), 456. https://doi.org/10.17628/ecb.2017.6.456-469

Bekro, Y.-A., Mamyrbekova, J. A., Boua, B. B., Bi, F. T., & Ehile, E. E. (2007). Étude ethnobotanique et screening phytochimique de Caesalpinia benthamiana (Baill.) Herend. Et Zarucchi (Caesalpiniaceae). Sciences & Nature, 4(2), Art. 2. https://doi.org/10.4314/scinat.v4i2.42146

Biner, B., Gubbuk, H., Karhan, M., Aksu, M., & Pekmezci, M. (2007). Sugar profiles of the pods of cultivated and wild types of carob bean (Ceratonia siliqua L.) in Turkey. Food Chemistry, 100(4), 1453-1455. https://doi.org/10.1016/j.foodchem.2005.11.037

Hajaji, H. E., Lachkar, N., Alaoui, K., Cherrah, Y., Farah, A., Ennabili, A., Bali, B. E., & Lachkar, M. (2011). Antioxidant activity, phytochemical screening, and total phenolic content of extracts

from three genders of carob tree barks growing in Morocco. Arabian Journal of Chemistry, 4(3), 321-324. https://doi.org/10.1016/j.arabjc.2010.06.053

Lachkar, N., Al-Sobarry, M., El-Hajaji, H., Lamkinsi, T., Lachkar, M., Cherrah, Y., & Alaoui, K. (2016). Anti-inflammatory and antioxidant effect of Ceratonia siliqua L. Methanol barks extract. Journal of Chemical and Pharmaceutical Research, 2016, 202-210.

Naghmouchi, S., Khouja, M. L., Romero, A., Tous, J., & Boussaid, M. (2009). Tunisian carob (Ceratonia siliqua L.) populations : Morphological variability of pods and kernel. Scientia Horticulturae, 121(2), 125-130. https://doi.org/10.1016/j.scienta.2009.02.026

Ronchetti, F., Russo, G., Bombardelli, E., & Bonati, A. (1971). A new alkaloid from Rauwolfia vomitoria. Phytochemistry, 10(6), 1385-1388. https://doi.org/10.1016/S0031-9422(00)84347-2

Sassi, A., Bouhlel, I., Mustapha, N., Mokdad-Bzeouich, I., Chaabane, F., Ghedira, K., & Chekir-Ghedira, L. (2016). Assessment in vitro of the genotoxicity, antigenotoxicity and antioxidant of Ceratonia siliqua L. extracts in murine leukaemia cells L1210 by comet assay. Regulatory Toxicology and Pharmacology, 77, 117-124. https://doi.org/10.1016/j.yrtph.2016.02.009

Wagner, H., Bladt, S., & Zgainski, E.-M. (2013). Drogenanalyse : Dünnschichtchromatographische Analyse von Arzneidrogen. Springer-Verlag.