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Comparative extraction techniques of alkaloids and evaluation of antioxidant capacity from the fruits of *Retama raetam*

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Abstract

Retama raetam is an important source of biologically active compounds, especially Piperidine and Quinolizidine alkaloids as it has been traditionally used in the treatment of many diseases. Our study aims to evaluate the extraction of alkaloids from different plant organs in different ways and to estimate DPPH free radical scavenging of total alkaloids and ethanolic extract from fruits. Alkaloids of *R. raetam* from Algerian Sahara was extracted using three different methods. Four parts of this plant were subjected to these extraction methods. The evaluation of the antioxidant activity was measured by using the free radical DPPH test. The highest stock of alkaloids was found in the stems followed by the roots and the lowest content was found in the fruits. Soxhlet extraction was the most efficient method to extract alkaloids from *R. raetam*. The extracts showed very interesting antioxidant activity. The percentages of DPPH inhibition are IC50= 0.087mg/ml and 0.097mg/ml respectively. Stem part of R. raetam was found to be the richer part in alkaloids. Fruit extract exhibited very interesting antioxidant activity.

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Introduction

Extraction is the separation of desired natural products from the raw material, and the commonest is solid-liquid extraction using solvents. Extraction is based on major steps; penetration of the solvent into the solid tissue of the plant (powder), the solute is dissolved from the natural compounds in the solvent, where the solubility increases with the convergence of the polarity of the solvent and the extracted solute, Diffusion of solute outside the solid plant and finally, collects the extracted solutions. Solvent selection is essential for extraction. Selectivity, solubility, cost and safety must be taken into account when selecting solvents, as the extraction efficiency is affected by the physical and chemical properties of the extraction liquid, solute(s), temperature and extraction time (Zhang *et al.*, 2018; Azmir *et al.*, 2013; Rostagno *et al.*, 2013; Alaraa *et al.*, 2021; Abah *et al.*, 2011). EtOH and MeOH are the most common solvents in extracting natural compounds. Alkaloids due to their acidic nature (salt) are better soluble in polar solvents and their basic (free) nature is better soluble in non-polar solvents and can be separated without any other secondary metabolites.

The following five steps are the most vital in separating and isolating alkaloids from their plant sources starting with the preparation of the plant sample by drying, and grinding appropriately then releasing the alkaloids to their basic nature (usually in the form of acidic salts in plants) using an appropriate base succeeded by the extraction of the basal alkaloids with a suitable solvent, After that it came the choice of an acceptable technique to obtain maximum yield, Finally we proceed to the purification of alkaloids from impurities by modifying the basic and acidic formula of alkaloids followed by the separation of alkaloids, according to their polarity, is usually separated by chromatography(Yubin *et al.*, 2014; Bruneton, 2016; Dey, 2020).

Retama raetam plant is spread in North Africa and the Middle East, where it grows in reefs, valleys, and desert areas. It is among the indigenous plants in southern Algeria. It has a major role in maintaining ecological balance and resisting desertification (Stavi *et al.*, 2010; Mittler *et al.*, 2001)

Retama raetam is known for its many uses in traditional medicine (Nur-e-Alam *et al.*, 2019; Chaachouay *et al.*, 2020), some of its biological and pharmacological properties are due to its varied content of alkaloids, especially Piperidine and Quinolizidine alkaloids (El-Shazly *et al.*, 1996; Hammouche-Mokrane *et al.*, 2017; Abdel-Halim *et al.*, 1992), these heterocyclic compounds are well known for their importance in pharmacology and toxicology (Keeler *et al.*, 1989; Ojima *et al.*, 1999; Kopp *et al.*, 2020 ; Li *et al.* 2020). The aim of the present work was to evaluate the extraction of alkaloids from different plant organs by Well-known extraction techniques, and to estimate the antioxidant activity (DPPH free radical scavenging) of total alkaloids and ethanolic extract from fruits.

Materials and methods

Plant material

The various parts of *Retama raetam* were obtained between February-April 2019 from the outskirts of Touggourt (Algeria). The plant was identified and authenticated with assistance of Y. Halis (laboratory of biochemistry, scientific and technical in the Research Center for Arid Areas, Touggourt, Algeria) for the identification of the plant material. The different parts of the plant (roots, stems, flowers, fruits) were dried in a ventilated place away from light, then pulverized, and kept in paper bags until use.

Extraction methods

Alkaloids were extracted in three different ways from different parts of the plant (roots, Stems, flowers, fruits).

The first method (alcoholic extraction)

The plant material is soaked in EtOH (96%), with gentle shaking for 24 hours, followed by filtering (three times). the combined extracts were concentrated until dry by rotary evaporator at 40°C. Then H_2SO_4 (0.25M) is added with mixing, then filtering. The collected filtrate is placed in a separating funnel; petroleum ether is added to it after that is extracted.

The petroleum ether loaded with impurities is separated, then ammonia is added until reach a basic

pH (pH = 9). Next, the chloroform is added to extract. This extraction process is repeated three times, and the chloroform extract is dried using anhydrousmgSO4. The anhydride chloroform extract is dried using rotary evaporator at 40°C, and its weight and yield are taken (Table 1).

The second method (acidified water extraction)

The plant powders are soaked in a solution of H2SO4 (0.25M), with gentle shaking for 24 hours followed by filtering. The process is repeated three times. The filtrate is placed in a separating funnel and we follow the same steps as in the first method and its weight and yield are taken (Table 1).

The third method (extraction using the Soxhlet device)

The various plant organ powders placed in cloth bags in a solution of NH3 (5M) for 24 hours. After that, each bag is placed in the Soxhlet apparatus, using CH2Cl2 as the solvent.

The extraction process was carried out until complete exhaustion (test for alkaloids), taking into account the gentle heating. After the extraction process is completed, the extract is dried by rotary evaporator, then a solution of H2SO4 (0.25M) is added to it.

The filtrate is placed in a separating funnel and we follow the same steps as in the first method and its weight and yield are taken (Table 1). The extract of fruit alkaloids for this third method (ALK) was preserved at 4 °C in storage vial for activity test.

Ethanolic extract from fruits

10 g of dried fruit powder was macerated (3 times) in ethanol (80%, 50mL) for 24 hours, after filtration, the combined extracts were concentrated until dry by rotary evaporator at 40°C, to give the ethanolic extract 0.830g. This ethanolic fruit extract (ETO) was preserved at 4°C in a storage bottle for Activity Assay.

DPPH Radical Scavenging Activity Assay

Extracts were dissolved in ethanol to prepare the concentrations ranging from 0.01 to 0.6mg/mL. For DPPH radical scavenging assay, 500 µL DPPH (0.004% prepared in ethanol) was added to 500 µL of a different concentration of crude extract sample. The reaction mixture was incubated for 30 min. The absorbance of the mixture was measured at 517 nm. The IP% (percentage of inhibitory) is calculated according to the formula:

% IP = [(Ato- At) / Ato× 100)]

Ato: absorbance of the control (containing no antioxidant) after 30 minutes

At: absorbance of extracts measured after 30 minutes

Results and discussion

Across the results (Table1) it is generally illustrated that the highest stock of alkaloids is found in the stems (0.36-1.50%) followed by the roots and the lowest in the fruits (0.16-0.28%). The highest percentage extraction was obtained when plant material is extracted with Soxhlet (Method 3, 1.50%) in all organs, and the lower percentage extraction with Alcoholic extraction (Method 1- Alcohol extraction) in all organs (Fig. 1).

Table 1. Yield values of the different extracts.

	Method 1- Alcohol extraction			Method 2- Extraction with acidified water			Method 3 - Soxhlet extraction		
Plant organ	Dry organ mass (g)	Alkaloids mass (g)	Yield R%	Dry organ mass (g)	Alkaloids mass (g)	Yield R%	Dry organ mass (g)	Alkaloids mass (g)	Yield R%
Roots	50	0.145	0.29	50	0.155	0.31	70	0.352	0.50
Stems	25	0.322	1.29	50	0.178	0.36	70	1.050	1.50
Flowers	25	0.050	0.20	100	0.215	0.21	70	0.168	0.24
Fruits	75	0.121	0.16	75	0.132	0.18	71	0.201	0.28

The high extractability of alkaloids (free) using the Soxhlet device are due to the good solubility of the alkaloids (free) of this plant in dichloromethane and heat, especially that the boiling point of this solvent (40°C) is smaller compared to other solvents, and therefore it is gentle on alkaloids that are easily broken by heat, such as esters. Kocanci *et al.* (2022) reported that using soxhlet gives the

best yield when comparing different protocols of alkaloid extraction.

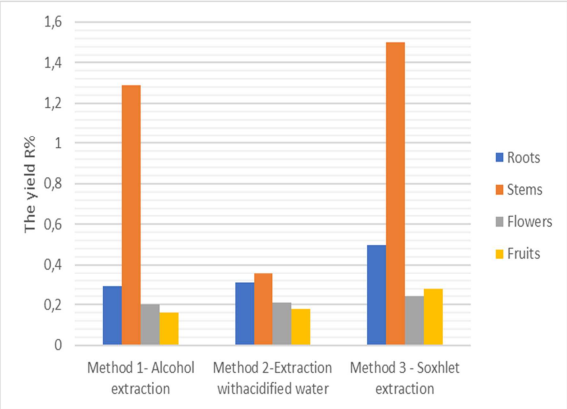

Fig. 1. Comparison between the alkaloids yield of the different plant organs.

Both ETO and ALK extracts from fruits showed very good antioxidant activity. ETO extract demonstrated a slightly more efficiency than ALK extract. The antioxidant activity (DPPH) of the alcoholic extract containing various classes of phytochemical compounds including the alkaloids is greater than that of the alkaloids extract containing only alkaloids (Table 2). Retama genus is known to contain flavonoids (Louaar, S., *et al.*, 2005) which are responsible of wide range of activities such as antioxidant (Ghani U., 2019). That may due to the affinity of different classes of phytochemical compounds including alkaloids with the used solvent. The convergence of the antiradical activity can be explained by the presence of alkaloids in both extracts and it's mainly responsible for the inhibition of the radical DPPH.

Table 2. IC50 of the antiradical activity.

extract	ETO	ALK
IC50	87.20 µg/ml	97.84 µg/ml

Conclusion

We conclude from this comparative study that the high percentage of alkaloids resides in the stems. We found also that the best way to extract alkaloids from this plant is to use the Soxhlet device. In addition of that, the fruit extracts showed very interesting antioxidant activity.

Conflict of interest

No, conflict of interest among all authors

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References

Abdel-Halim OB, Sekine T, Saito K, Halim AF, Abdel-Fattah H, Murakoshi I. 1992. (+)-12α-Hydroxylupanine, a lupin alkaloid from Lygos raetam. Phytochemistry **40,** 3251-3253. doi:10.1016

Abah SE, Egwari LO. 2011. Methods of Extraction and Antimicrobial Susceptibility Testing of Plant Extracts. African Journal of Basic & Applied Sciences **3(5),** 205-209. https://core.ac.uk

Al-Rehaily AJ. 2019. New Flavonoids from the Saudi Arabian Plant *Retama raetam* which Stimulate Secretion of Insulin and Inhibit α-Glucosidase. Organic & Biomolecular Chemistry **17,** 1266-1276.

Alaraa OR, Abdurahmana NH, Ukaegbu CI. 2021. Extraction of phenolic compounds: A review. Current Research in Food Science **4,** 200-214. DOI: 10.1016/j.crfs.2021.03.011

Azmir J, Zaidul ISM, Rahman MM, Sharif KM, Mohamed A, Sahena F, Jahurul MHA, Ghafoor K, Norulaini NAN, Omar AKM. 2013. Techniques for extraction of bioactive compounds from plant materials: A review. Journal of Food Engineering **117(4),** 426-436.

Bruneton J. 2016. Pharmacognosie, phytochimie, plantes médicinales, 5éd, Éditions Lavoisier Tec & Doc. DOI: 10.1007/S10298-017-1173-5

Chaachouay N, Benkhnigue O, Zidane L. 2020. Ethnobotanical Study Aimed at Investigating the Use of Medicinal Plants to Treat Nervous System Diseases in the Rif of Morocco. Journal of Chiropractic Medicine **19(1),** 70-81.

Dey P, Kundu A, Kumar A, Gupta M, Lee BM, Bhakta T, Dash S, Kima HS. 2020. Analysis of alkaloids (indole alkaloids, isoquinoline alkaloids, tropane alkaloids). Recent Advances in Natural Products Analysis 505–567.
DOI: 10.1016/B978-0-12-816455-6.00015-9

Dey P. 2020. (ed.): Recent Advances in Natural Products Analysis, Analysis of alkaloids (indole alkaloids, isoquinoline alkaloids, tropane alkaloids), Elsevier Publishing pp. 505.
DOI: 10.1016/b978-0-12-816455-6.00015-9

El-Shazly A, Ateya AM, Witte L, Wink M. 1996. Quinolizidine Alkaloid Profiles of *Retama raetam*, R. sphaerocarpa and R. monosperma. Zeitschrift für Naturforschung **51c,** 301-308. DOI: 10.1515/ZNC

Ghani U, Nur-e-Alam M, Yousaf M, Ul-Haq Z, Noman OM, Al-Rehaily AJ. 2019. Natural flavonoid α-glucosidase inhibitors from *Retama raetam*: Enzyme inhibition and molecular docking reveal important interactions with the enzyme active site. Bioorganic Chemistry **87 (6),** 736-742.
DOI: 10.1016/J.BIOORG.2019.03.079

Hammouche-Mokrane N, León-González AJ, Navarro I, Boulila F, Benallaouad S, Martín-Cordero C. 2017. Phytochemical Profile and Antibacterial Activity of *Retama raetam* and R. sphaerocarpa cladodes from Algeria. Natural Product Communications **12(12),** 1857-1860.
DOI: 10.1177 /1934578X1701201211

Keeler RF, Panter KE. 1989. Piperidine Alkaloid, Composition and Relation to Crooked Calf Disease-Inducing Potential of Lupinus formosus. Teratology **40(5),** 423-432. DOI: 10.1002/tera.1420400503

Kocanci FG, Dolanbay SN, Aslim B. 2022. Comparison of three different protocols of alkaloid extraction from Glaucium corniculatum plant. International Journal of Secondary Metabolite **9(1),** 43-51.
DOI: 10.21448/ijsm.980171

Kopp T, Abdel-Tawab M, Mizaiko B. 2020. Extracting and Analyzing Pyrrolizidine Alkaloids in Medicinal Plants: A Review. Toxins **12(320),** 320.

Louaar S, Akkal S, Bousetla A, Medjroubi K, Djarri L, Seguin E. 2005. Phytochemical study of Retama sphaerocarpa. Chemistry of natural compounds **41(1), 107**-108.

Li Y, Wang G, Liu J, Ouyang L. 2020. Quinolizidine alkaloids derivatives from Sophora alopecuroides Linn: Bioactivities, structure-activity relationships and preliminary molecular mechanisms. European Journal of Medicinal Chemistry **188(2020),** 111972. DOI: 10.1016/ j.ejmec

Mittler R, Merquiol E, Hallak-Herr E, Rachmilevitch S, Kaplan A. 2001. living under a 'dormant' canopy: a molecular acclimation mechanism of the desert plant *Retama raetam*. The plant journal **25(4),** 407- 416.
DOI: 10.1046/ J.1365-313x.2001. 00975.X

Manske RHF, Holmes HL. 1950. (eds): The Alkaloids, Chemistry and Physiology, Chapter I, Sources of Alkaloids and their Isolation, Elsevier Publishing, **1,** pp1.
DOI: 10.1016/S1876-0813(08) 6018

Norulaini NAN, Omar AKM. 2013. Techniques for extraction of bioactive compounds from plant materials: A review; Journal of Food Engineering **117,** 426.
DOI: 10.1016/j.jfoodeng.2013.01.014

Nur-e-Alam M, Yousaf M, Parveen I, Hafizur RM, Ghani U, Ahmed S, Hameed A, Threadgill MD, Ojima I, Iula DM. 1999. Chapter Five - New Approaches to the Syntheses of Piperidine, Izidine, and Quinazoline Alkaloids by Means of Transition Metal Catalyzed Carbonylations. Alkaloids: Chemical and Biological Perspectives. Department of Chemistry State University of New York at Stony Brook 371-412.
DOI: 10.1016/S0735-8210(99)80028-4

Rostagno MA, Prado JM. 2013. (eds.): Extraction of natural products, principles and fundamental aspects. In Natural Product Extraction, Principles and Applications, RSC Publishing, Cambridge.

Stavi I, Perevolotsky A, Avni Y. 2010. Effects of gully formation and headcut retreat on primary production in an arid rangeland: Natural desertification in action. Journal of Arid Environments **74(2),** 221-228.
DOI: 10.1016/j.jaridenv.2009.08.007

Yubin JI, Miao Y, Bing W, Yao Z. 2014. The extraction, separation and purification of alkaloids in the natural medicine. Journal of Chemical and Pharmaceutical Research **6(1),** 338-345. http://jocpr.com/vol6-iss1-2014/JCPR

Zhang QW, Lin LG, Ye WC. 2018. Techniques for extraction and isolation of natural products: A comprehensive review. Chinese. Medicine **13(1),** 1-26. DOI: 10.1186/s13020-018-0177-x