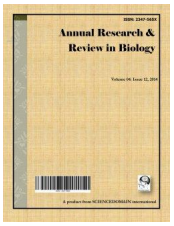

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New Source of Rutin from the Flowers of *Cordia lutea (Boraginaceae)*

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Authors' contributions

This work was carried out in collaboration between all authors. Authors EAVC and CBKS designed the study, wrote the protocol, and wrote the first draft of the manuscript. All authors managed the analyses of the study including three experimental proceses to validate the methodology used. Author ACC developed structural analysis. Author SMBC managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The native species *Cordia lutea*, is used in Peru by folk medicine in the treatment of liver diseases such as jaundice and others. From fluid extract of the flowers, flavonoid quercetin-3-O-rhamnoglucoside (rutin) was isolated and identified. The relative purity was assay by HPLC and structural elucidation using ^{1}H and ^{13}C NMR spectral data in comparison with literature. Quercetin-3-O-rhamnoglucoside (rutin), was the major component of the fluid extract obtained from *C. lutea* flowers, with a relative purity, after crystallization, of 97.55% and 8% yield from the extract.

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As a conclusion for this investigation, it is important to note, that with the fluid extract from the flowers of C. lutea it is possible the isolation of the flavonoid quercetin-3-O-rhamno glucoside (rutin) with high purity and yield. This compound was not previously described in the flowers of this plant species.

Keywords: Cordia lutea flowers; Rutin.

1. INTRODUCTION

Cordia lutea is a plant belonging to *Boraginaceae* family. Is a plant that grows 7.5 m of high, the bark is dark brown, fissured, is a branched tree with campanulate flowers yellow in color. The plant growths in the north of Peru, in the regions of Tumbes, Piura, Cajamarca, Lambayeque and La Libertad.

The flower of *C. lutea* (Fig. 1) are corolla bell (gamopetalous) type, tubular chalice; 4 sepals, stamens 9:1 carpel and a single petal. Has an average weight of 0.1127 g, 2.3 cm wide and 2.9 cm long. Within the organoleptic characteristics it is a yellow flower, with “sui generis” odor, slightly bitter flavor and smooth consistence. Literature refers the presence of terpenoids and phenolic compounds in the plant. [1].

Fig. 1. *Cordia lutea* flowers

The flowers are used in traditional medicine, in the form of decoctions for the treatment of liver diseases in particular jaundice [1]. Chemical composition of the extracts from the flowers, suggest the presence of phenolic compounds, in particular flavonoid glycosides, rutin, according to our results.

The natural source for rutin, in literature references, are the flowers of *Ruta graveolens*, the rind of citric fruits and in the flowers of *Sambucus nigrans* and *Sambucus peruviana* H.B.K. [2-4]. Rutin is very important in medicine because it’s anti-inflammatory, antispasmodic and its hepatic protector activity, to avoid capillary fragility and in particular for its antioxidant activity and as a potential anticancer flavonoid [5].

Those activities increase the demand of the flavonoid in pharmaceutical trade, and for that, it is important to find some new natural sources for the possible industrial production of this flavonoid glycoside [6].

Our group is evaluating the yield of this flavonoid glycoside from different sources during some time, in particular the flowers of *C. lutea,* in order to standardize a methodology to obtain a high yield of the flavonoid.

2. MATERIALS AND METHODS [7- 9]

2.1 Plant Material

The flowers of *C. lutea* were collected in Cajamarca, Peru, in October 2017. The flowers were authenticated by Dr. José Mostacero, Director of Truxillensis Herbarium, National University of Trujillo, number of Voucher 33425.

2.2 Methodology for the Extraction

Each 100 g of the flowers of *C. lutea were* dried in stove during 50 hours to 40ºC and reduced by milling to particle size 5 mm after draying. The plant material moistening with 200 mL of ethanol-water 50% vol/vol during 45 minutes, introduced into a percolator equipment for the preparation of a fluid extract.

2.3 Fluid Extract

Each 100 mL of fluid extract, were put in a refrigerator during 2 hours and let it stand until the precipitation of the flavonoid glycoside.

The solid obtained is recrystallized from water to obtain the product with the quality necessary for structural elucidation.

2.4 Determination of the Purity of the Compound by *High-Performance Liquid Chromatography (HPLC)*

0.001 g of the product recrystallized, dissolved in 5 mL of methanol (Merck), grade HPLC, was deposited in an equipment Agilent 1100 with photodiode array detector, calibrate to 256 nm, volume of injection of 10 uL, running time 5 minutes to do the determination.

2.5 Spectral Analysis

2.5.1 Nuclear Magnetic Resonance (NMR^{1}H) y (NMR^{13}C)

Spectral data was obtained using a Brücker AC 300 MHz equipment with Chloroform-d as solvent and tetra methyl silane as internal reference.

3. RESULTS

The results of the purity and spectral analysis of the product isolated are shown Fig. 2.

4. DISCUSSION

Before the preparation of the fluid extract, the flowers were evaluated for some pharmacognostic parameters necessary for the quality of the plant material as a source for the production of the flavonoid rutin.

The fluid extract obtained from the flowers of *C. lutea* was orange in color, pH between 4,5-5 and bears a bitter taste. Phytochemical screen of the extract, gave positive results for the following assays: phenols, flavonoids, triterpenoids, lipids, foaming and reducing agents.

Total solids components in the extract was 321 mg/mL.

After refrigeration, at least during 24 hours, precipitation occur, the solid was recuperated by filtration with vacuum, weighing 8 grams in average per each 100 mL of extract (8%)

The solid was recrystallized from water to obtain a white product that was analyze to state the purity and the chemical structure.

HPLC analysis (Fig. 2, Table 1) shows a purity on 97, 55% for the solid obtained, so it was analyzed through NMR spectroscopy for structural elucidation.

NMR-^{1}H spectral data (Fig. 3, Table 2) indicate the presence of rhamnose, the signal of 3, 28 ppm is indicative of the sugar in a flavonoid glycoside. The presence of free phenolic hydroxides is confirmed through the signals at C-2' (7, 62 ppm), C-6' (7, 63 ppm) and C-5' (6, 87 ppm) coincident with the ones for quercetin nucleus.

NMR -^{13}C shows 27 signals for carbon atoms and a complete coincidence with the reports for rutin structure (Fig. 4, Table 3) [10]. The total assignments of the spectral analysis data are shown in Fig. 5.

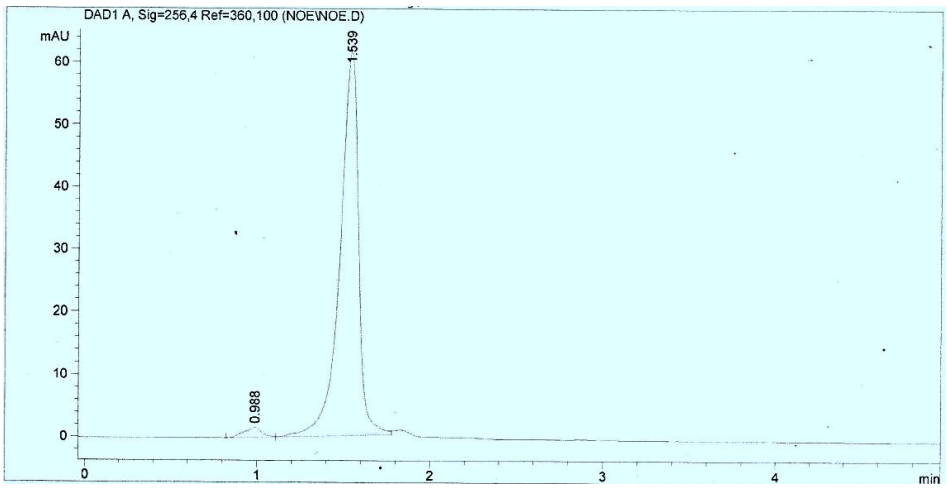

Fig. 2. High-performance liquid chromatography (HPLC) of the product recrystallized

Table 1. Determination of the relative purity by *High-performance liquid chromatography (HPLC)*

Signal N°	Retention Time	Type	Witdh [Min]	Area [Mau*s]	High [Mau]	Area %
1	0,988	PV	0,0970	11,58473	1,62749	2,4444
2	1,539	VB	0,1133	462,34839	61,80511	97,5556
Total				473,93312	6,343,260	

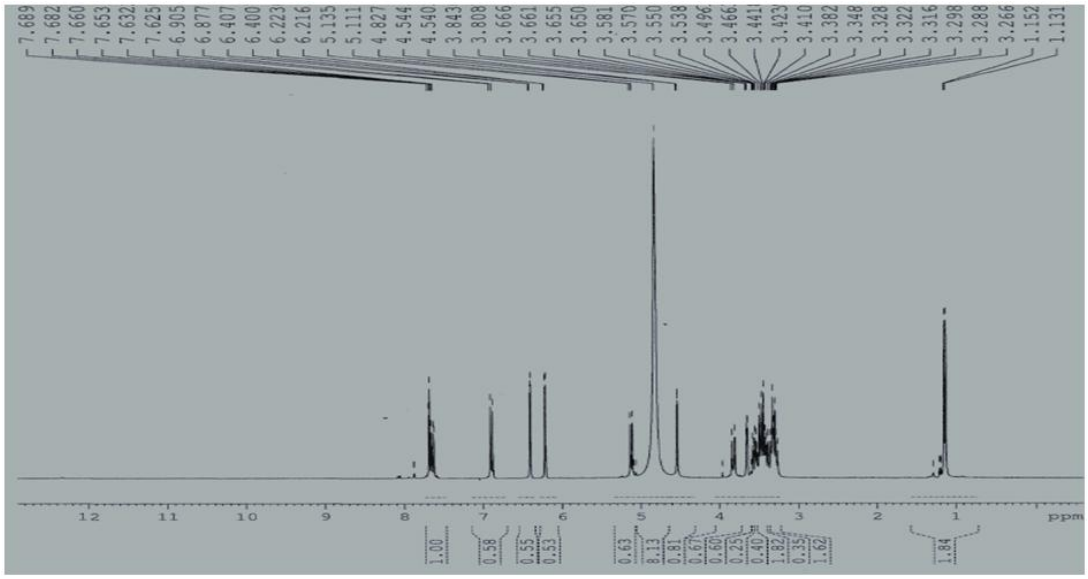

Fig. 3. NMR-^{1}H of the product recrystallized

Table 2. NMR -^{1}H spectral data of the product recrystallized

Aglycon	Protons	Quercetin	Product Isolated
	6	6,28	6,22
	8	6,38	6,40
	2'	7,60	7,62
	3'	-	-
	5'	6,84	6,87
	6'	7,60	7,63
Carbohydrate 1		**Glucose**	
	1	4,52	4,54
	2	3,52	3,53
	3	3,47	3,46
	4	3,42	3,42
	5	3,35	3,34
	6	1,15	1,15
Carbohydrate 2		**Rhamnose**	
	1	5,10	5,11
	2	3,27	3,26
	3	3,31	3,31
	4	3,45	3,44
	5	3,28	3,28
	6	3,56/3,79	3,55

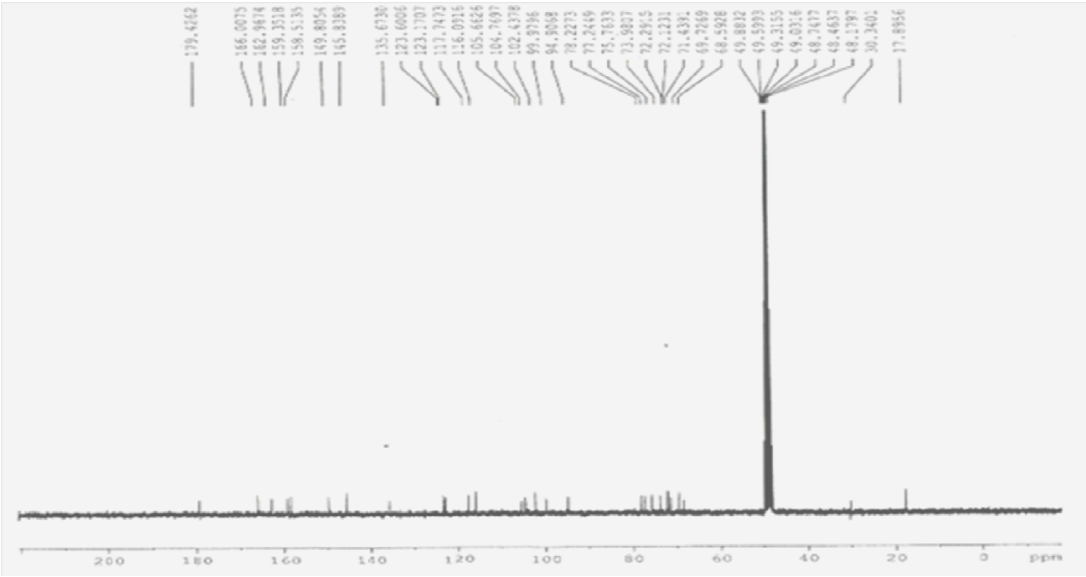

Fig. 4. NMR-^{13}C of the product recrystallized

Table 3. *NMR -^{13}C.* spectral data of the product recrystallized

Aglycon	Carbon	Quercetin	Product Isolate
	2	158,36	158,51
	3	135,61	135,67
	4	179	179,42
	5	162,74	162,96
	6	99,95	99,97
	7	165,83	166,00
	8	94,89	94,9
	9	159,25	159,35
	10	105,9	105,66
	1'	123,12	123,17
	2'	117,73	117,74
	3'	145,67	145,83
	4'	149,67	149,8
	5'	116,05	116,09
	6'	123,57	123,6
Carbohydrate 1		**Glucose**	
	1	104,76	104.76
	2	78,14	78,22
	3	73,93	73,98
	4	72,02	72,12
	5	75,68	75,76
	6	68,52	68,59
Carbohydrate 2		**Rhamnose**	
	1	102,23	102,43
	2	71,37	71,43
	3	72,26	72,29
	4	77,12	77,24
	5	69,63	69,72
	6	17,82	17,89

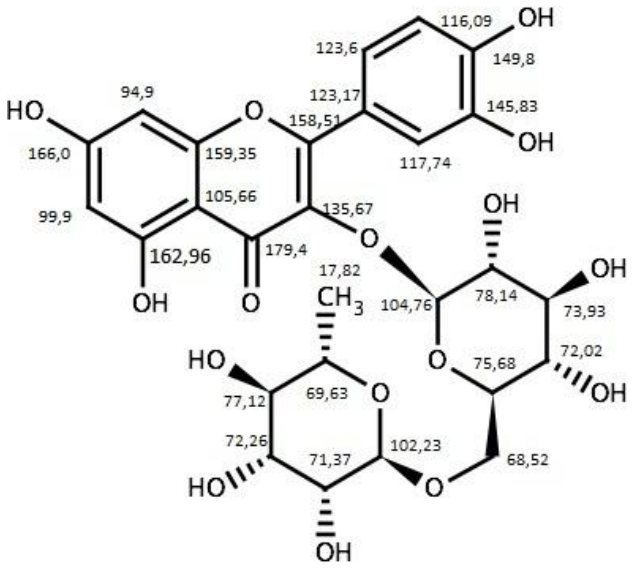

Fig. 5. Experimental data assignations for the product recrystallized according to NMR -^{13}C

5. CONCLUSION

As a conclusion for this investigation, it is important to note, that with the fluid extract from the flowers of *C. lutea* it is possible the isolation of the flavonoid quercetin-3-O-rhamno glucoside (rutin). This compound was not previously described in the flowers of this plant species. the relative purity of the product was assay by HPLC and structural elucidation using ^{1}H and ^{13}C NMR spectral data in comparison with literature. Quercetin-3-O-rhamnoglucoside (rutin), was isolated and characteraized as the main flavonoid component in *C. lutea* fluid extract from the flowers. The flavonoid after one recrystallization from water had a relative purity of 97.55% and a yield of 8% from the fluid extract prepared from the dried flowers.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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