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GC-MS analysis and antiacetylcholinesterase activity of the dichloromethane leaves extract of *Ravenia spectabilis* Lindl. (Rutaceae)

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Abstract: *Ravenia spectabilis Lindl. is a plant that belongs to the genus Ravenia (Rutaceae). It is a tall, medium-sized shrub that occurs widely in South America, and can also be found in India, Pakistan, Bangladesh and other countries in Asia. Previous phytochemical studies reported the presence of quinolone alkaloids, pyranocoumarins, flavonoids and various bioactive terpenes in its aerial parts. Antibacterial and cytotoxic activities are also reported for extractives from this plant. Dichloromethane leaves extract of a specimen cultivated in the state of Rio de Janeiro, Brazil, was analyzed by GC-MS and led to the identification of the compounds isatin, lichexanthone and the terpenes α-spinasterone, lupeol, sitostenone and α-cadinol in its composition. Some of them are found for this time in both genus Ravenia and R. spectabilis. Dichloromethane leaves extract was also tested for acetylcholinesterase (AchE) inhibition and showed 54.4± 3.70 (0.1 mg/mL) % of activity.*

Keywords: *Ravenia spectabilis, GC-MS, bioactive compounds, AChE inhibition*

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I. Introduction

Ravenia spectabilis (Rutaceae) is a versatile shrub widely spread throughout South America and some Asian countries such as India, Pakistan and Bangladesh. Previous chemical studies of leaves extracts reported the accumulation of quinolone alkaloids such as arborinine[1,2], ravenine [2,3], ravenoline [2,3] and atanine [2]. Quinolone alkaloid ravesilone, paraensin and acridone alkaloids were also isolated from leaves[4,5]. In 2013, in addition to these metabolites, other compounds were isolated from its stems such as the steroids stigmasterol and spinasterone, alkaloid γ-fagarine, geranyl indole and 3-methoxy-4-hydroxy cinnamic acid[6]. Concerning to biological activities, literature reports only antimicrobial and cytotoxic properties for extractives of this plant[7,8] and most of the articles presented studies of specimens from Asia, more specifically plants from Bangladesh. The main goal of this paper is to present the results of a GC-MS analysis of a dichlormethane leaves extracts of a specimen of *R. spectabilis* cultivated in Brazil aiming to compare its chemical profile with the literatures, what might work even as a search for bioactive compounds. Additionally, the extract was tested for *in vitro* inhibition of the enzyme acetylcholinesterase, a key enzyme involved in Alzheimer´s Disease, which is becoming a great concern all over the world as population grows older every year.

II. Material And Methods

2.1 Plant material

Leaves of *R. spectabilis* were collected at the Botanical Garden of Rio de Janeiro (Rio de Janeiro, Brazil) in August, 2016 and identified by Dr. Marcelo Trovó, from the Institute of Biology of the Federal University of Rio de Janeiro (Rio de Janeiro, Brazil). A voucher specimen is deposited at the Herbarium of the Botanical Garden of Rio de Janeiro under the code Giordano, LC 901 (RB).

2.2 Obtention of the extract

Fresh plant material was dried in an oven under the temperature of 40º C for 48 hours. Dried material was grounded to a fine powder in a mill and this powder was submitted to an extraction by maceration with dichloromethane (USP Grade) for 5 days. Solvent was further evaporated under vacuum and the residue was stored at room temperature under Nitrogen atmosphere.

2.3 GC-MS analysis

Dichloromethane leaves extract was analyzed by GC-MS at the Analytical Center of the Instituto de Pesquisa de Produtos Naturais of the Universidade Federal do Rio de Janeiro, Rio de Janeiro city, in Brazil. 1 mg of the extract was ressuspended in 1mL dichloromethane (HPLC grade) and filtered through a Sep-pak cartridge to yield. 1 mL of this solution was injected in a Shimadzu GC-2010 equipped with a DB5-MS column (30 m x 0.25 mm I.D. x 0.25 micro mdf) and coupled with a quadrupole detector. Programmed temperature for the oven temperature was initiated at 70 ^{o}C, increasing 3.0^{o}C /min to reach 260^{o}C and kept at this temperature for 5 min, then from 280 to 300^{o}C (3^{o}C / min, holding for 2 min); injector temperature 290^{o}C. Column flow was 1.00mL/min. Helium was used as carrier gas and ionization energy of 70eV. The percent relative amount of each component was calculated by comparing their average peak area to total area. Analysis was performed in triplicate to achieve more accurate results.

2.4 Acetylcholinesterase inhibition assay

Dichloromethane leaves extract of *R. spectabilis* was submitted to an evaluation of its anticholinesterase activity using the 96-well plate assay based on an adapted Ellman´s method [9]. Enzyme Acetylcholinesterase (AChE, from electric eel-type VI-S) and reagents acetylthiocholine iodide (ATCI), ,5,5´-dithiobis[2-nitrobenzoic acid] (DTNB) and bovine serum albumin (BSA) were all purchased from Sigma-Aldrich (St. Louis, MO, USA) and properly stored until their use. In a well of the microplate, 20 uL of 1.0 mM ATCI, 55 µL of 0.1 M phosphate buffer (pH 8.0), 100 µL of a solution 1 mg/mL of the extract (sample) dissolved in buffer containing 5% MeOH, and 25 uL of 0.25 U/mL of the enzyme were mixed. Reaction was maintained at 30ºC for 20 min in a water bath and 100 µL of a solution 1 mg/mL of the extract sample (dissolved in buffer containing 5% MeOH) was added to the well. Additionally, as a negative control, a well containing buffer instead of the substrate solution as also incubated in the same conditions. Enzyme activity was then monitored nm in a microplate reader Biotek® at 412 nm every 20s during 4 min. Enzyme activity was calculated as the percentage of velocity compared to that of the assay using buffer without any inhibitor. Assays were done in triplicate.

III. Results and Discussion

3.1 GC-MS analysis

GC-MS analysis revealed the presence of six major compounds in the dichoromethane leaves extracts of *R. spectabilis*, corresponding to 80.07% of its composition. Other substances in the chromatogram could not be identified because their peaks, in the chromatogram, led to unconclusive MS spectra. Substances identified were: indol derivative isatin, sesquiterpene α-cadinol, lichenxanthone, steroids spinasterone and sitostenone and the triterpene lupeol. From these, only sitostenone and spinasterone were found previously in phytochemical studies of *R. spectabillis*. Isatin and lichexanthone are reported for the first time for both genus *Ravenia* and the species. Lupeol and α–cadinol are reported for the first time in the species. Chromatogram of the GC analysis is shown in **Figure 1** and **Table 1** brings the data concerning to the peaks related to each substance identified by GC-MS results.

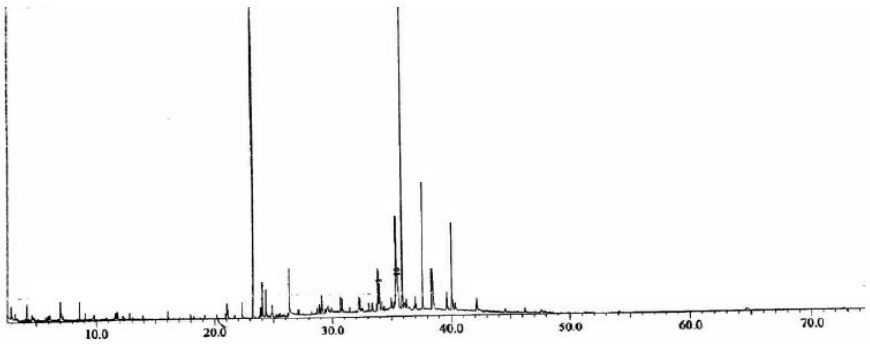

Figure 1- Gas chromatography-mass spectrometry chromatogram of the dichloromethane leaves extract of *R.* spectabilis

Table 1 – Constituents of dichoromethane leaves extract of *R. spectabilis* according to the GC-MS analysis.

	Compound	**MW (g/mol)**	**Formula**	**RT (min)**	**Area (%)**
1		147,13	$C_8H_5NO_2$	23,24	24,82
2		222,37	$C_{15}H_{26}O$	26,30	3,79
3		286,28	$C_{16}H_{14}O_5$	35,40	9,36
4		410,68	$C_{29}H_{46}O$	35,92	24,28
5		417,70	$C_{29}H_48O$	37,66	9,82
6		426,72	$C_{30}H_{50}O$	40,04	8,00
				Total (%)	**80,07**

Although quinolone alkaloids are routinely found in phytochemical studies of *R. spectabilis*, no one of them was detected in this analysis. As the botanical identification left no doubt about the identity of the specimen, it is suggested that these alkaloids might be rather found in more polar extracts such as ethanol or methanol from diverse parts of the plants (leaves, stems, roots).

Most of the substances identified in this extract can be considered bioactive, according to literature. Isatin, an indol derivative, is a substance found in the genus *Isatis* of the plant family Brassicaceae and it is very useful in synthesis of Schiff bases. Several of these bases are reported to have antibacterial[10], antifungal[10] and antiviral[11] activities among others. Sesquiterpene α-cadinol is more likely to be found in plants as a constituent of volatile oils. Many species whose essential oils are biologically active, revealed this sesquiterpene as one of their constituents, such as some Phillipine plants[12]. Lupeol is a very bioactive triterpene and a review upon its activities was published in 2011[13]. This review reports some interesting properties for this terpene as anti-inflamatory and anti-arthiritic activities among others. Anti-cancer activity is another important activity recently related to lupeol[14]. It was not found in literature any significant biological activity for the steroids sitosten-3-en-4-one and spinasterone alone, despite of their significant presence in many plant extracts. Lichenxanthone is a substance more commonly produced by lichens, but also found in plants such as *Minquartia guianensis*[15] and

Zanthoxylum tetraspermum[16]. There are not many reports of biological activities for this xanthone besidest antimycobacterial[17] and antifungal[18] ones when it was found in lichen.

3.2 Acetylcholinesterase inhibition

Dichlorometane leaves extract of *R. spectabilis* presented a 54.4% ± 3.70 of AChE inhibition according to the Ellman assay. This inhibition is comparable to the ones reported for other plant extracts from Rutaceae such as for fruits of *Aegle marmelos* (44,6% ±0.3)[19] and leaves of *Esenbeckia leioacarpa* (91,1%±0.2)[20]. This is a preliminary study of AChE inhibition and IC_{50} of extract and fractions from the further chromatographic procedures must be achieved. IC_{50} values will be necessary to compare with the AChE inhibitors traditionally used in Alzheimer´s Disease treatment such as galanthamine and physostigmine. However it is a promising result, specially because the extract is a complex mixture of substances specially terpenes that literature indicates as potential AChE inhibitors[21].

IV. Conclusion

This new phytochemical study by GC-MS of leaves from *R. spectabilis* led to the identification of six natural products. Out of these, four compounds have never been described for this plant and two are new to the genus *Ravenia*. Leaves of this plant proved to be an important source of bioactive natural products as some of the identified compounds, such as isatin and lupeol. Acetylcholinesterase inhibition by the dicloromethane leaves extract was promising and might be related to one or more compounds present in this mixture. Substances will be isolated from the extract and have their IC_{50} for this AChE inhibition tested and compared to the standards, which are the commercially available drugs used in Alzheimer´s disease treatment.

References

[1]. Das KC, Bose Pk. Alkaloids from *Ravenia spectabilis*. Trans. Bose Res. Inst. (Calcutta). 1963; 26(4): 129-130.
[2]. Paul BD, Bose PK. Quinolone alkaloids from *Ravenia spectabilis.* Indian J. Chem. 1969; 7(7): 678-680.
[3]. Paul BD, Bose Pk. New quinolone alkaloids from *Ravenia spectabilis*. J. Indian Chem. Soc. 1968; 45(6): 552-553.
[4]. Battacharyya P, Chowdbury BK. Ravesilone, a quinolone alkaloid from *Ravenia spectabilis*. Phytochemistry. 1984; 23(8): 1825-1826.
[5]. Khan MA, Waterman PG. Constituents of *Ravenia spectabilis*. Fitoterapia. 1990; 61(3); 282
[6]. Hague, Md M, Begum S, Sohrab Md. H, Ahsan M, Hasan CM, Ahmed N; Hague R. Secondary metabolites from the stem of *Ravenia spectabilis* Lindl. Pharmacog. Mag. 2013; 9(33): 76-80.
[7]. Alam A, Asaduzzaman M, Nasrin N. Isolation, characterization and evaluation of Anti-Microbial activity of *Ravenia spectabilis* (Rutaceae). Int. J. of Pharmacog. and Pharm, Res. 2011; 393): 80-84.
[8]. Sohrab MH, Chowdbury R, Rahman KM, Hasan CM, Rashid MA. Antibacterial activity and citotoxicity of extractives from *Ravenia spectabilis*. Fitoterapia. 2004; 75(5): 510-513.
[9]. Ellman GL, Lourtney DK, Andres V, Gmelin G. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 1961; 7: 88–95.
[10]. Kupinìc M, Medic-Skaric M, Moorin M, Duskica M. Antibacterial and antifungal activities of isatin N-Mannich bases. J. Pharm. Sci. 1979; 68(4): 459-462.
[11]. Jarrahpour A, Khalili D, DeClerq E, Salmi C, Brunel JM. Synthesis, antibacterial, antifungal and antiviral activity evaluation of some new Bis-Schiff Bases of isatin and their derivatives. Molecules. 2007; 12(8): 1720-1730.
[12]. Juliani HR. Physical and chemical properties, composition and biological activity of Essential Oils of Phillippine medicinal plants. J. Med. Act. Plants. 2017; 5(2): 28-35.
[13]. Wal P, Wal A, Sharma G, Rai A. Biological activities of lupeol. Syst. Rev. in Pharm. 2011; 2(2): 96-103.
[14]. Magalhães GC, Duarte LP, Mussel WN, Ruiz ALTG, Shiozawa L, De Carvalho JE, Trindade IC, Vieira-Filho SA. Lupeol and its esters: NMR, powder XRD data and *in vitro* evaluation of cancer cell growth. Braz. J. Pharm. Sci. 2017; 53(3): e00251.
[15]. El-Seedi HR, Hazell AC, Torssell KBG. Triterpenes, lichexanthone and an acetylenic acid from *Minquarica guianensis.* Phytochemistry. 1994; 35(5): 1297-1299.
[16]. Nissanka APK, Karunaratne V, Bandara BMR, Kumar OV, Tsutomu N, Nishi M, Inada A, Tillekeratne LMV, Wijesundara DSA, Gunatilaka LAA. Antimicrobial alkaloids from *Zanthoxylum tetraspermum* and *caudatum*. Phytochemistry, 2001; 56(8): 857-861.
[17]. Michelletti AC, Honda HN, Pavan FR, Leite CQF, Matos MFC, Perdomo RT, Bogo D, Alcântara GB, Beatriz A. Increment of antimycobacterial activity on lichexanthone derivatives. Med. Chem. 2013; 9: 904-910.
[18]. Goel M, Dureja P, Rani A, Uniyal PL, Laatsch H. Isolation, characterization and antifungal activity of major constituents of the Hymalayan lichen *Parmelia reticulata* Tayl. J. Agric. Food Chem. 2011; 59(6): 2299-2307.
[19]. Ingkanninan K, Temkithawon P, Chuenchon K, Yuyaem T, Thongnoi W. Screening for acetylcholinesterase inhibitory activity in plants used in Thai traditional rejuvenating and neurotonic remedies. J. Ethnopharmacol. 2003; 89: 261-264.
[20]. Cardoso-Lopez EM, Maier JA, Da Silva MR, Regasini LO, Simote SY, Lopes NP, Pirani JR, Bozani VS, Young MCM, Alkaloids from *Esenbeckia leiocarpa* Engl. (Rutaceae) as potential treatment for Alzheimer Disease. Molecules. 2010; 15(12): 9205-9213.
[21]. Sauvaître T, Barlier M, Herlem D, Gresh N, Chiaroni A, Guenard D and Guillou C. New Potent Acetylcholinesterase Inhibitors in the Tetracyclic Triterpene Series. *J. Med. Chem.* 2007; *50* (22): 5311–5323.

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