Bioprospecting *Chukrasia tabularis* A. Juss. leaves: HPLC-DAD-ESI/MS^n profiling of phenolic bioactives and effects on inflammatory mediators and enzymatic targets engaged in metabolic disorders

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INTRODUCTION

Despite the global investigational efforts, metabolic disorders, particularly obesity and diabetes, remain a worldwide pandemic that continues to grow at an alarming level, as the number of cases and their prevalence have been steadily increasing over the past few decades [1]. Such paradigm comprises significant health and economic costs for governmental entities, that call for a global revitalization of healthier life-styles and the systematic investigation of new herbal medicines. *Chukrasia tabularis* A. Juss. is a canopy tree mainly found in India, China, Sri Lanka and Thailand, used in traditional medicine as an antipyretic, astringent and antidiarrheal agent [2]. Notwithstanding its widespread distribution in the Asian territory, the plant has been recently included in The International Union for Conservation of Nature's Red List of Threatened Species, mostly due to wood over-exploration [3]. In this regard, fostering the 3 Rs policy (Reduce, Reuse & Recycle) and aiming the valorisation of *C. tabularis* waste, we investigated the biological/pharmacological effects of the leaves. We selected a panel of mediators and enzymatic targets underlying the pathogenesis and development of diabetes and other metabolic disorders. To further broaden the current knowledge on the chemical composition of *C. tabularis* leaves and to identify bioactives that might contribute to the recorded pharmacological effects, the polyphenolic profile of the methanol extract was characterized by HPLC-DAD-ESI/MS^n.

METHODOLOGY

CHARACTERIZATION OF PHENOLIC PROFILE
- → **Qualitative analysis** | HPLC-DAD-ESI/MS^n
- → **Quantitative analysis** | HPLC-DAD

BIOLOGICAL/PHARMACOLOGICAL EFFECTS
- → **Enzymatic inhibition** | α-amylase, α-glucosidase, aldose reductase, pancreatic lipase and 5-LOX
- → **Antiradical activity** | DPPH$^•$, $^•$NO, $O_2^{•-}$, lipid peroxidation
- → **Modulation of inflammatory mediators in LPS-challenged RAW 264.7 macrophages** | NO, L-citrulline, IL-6 and TNF-α

RESULTS

Phenolics characterization

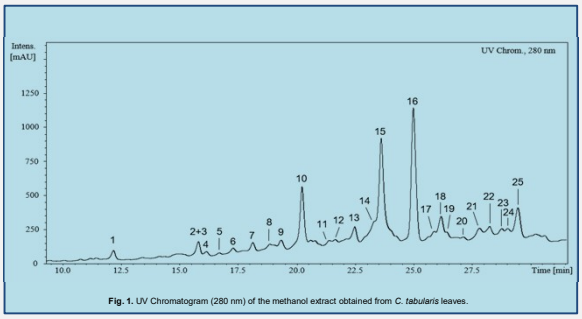

Fig. 1. UV Chromatogram (280 nm) of the methanol extract obtained from *C. tabularis* leaves.

Table 1.- *Rt*, UV, molecular formula, [M-H]$^-$, MS^2[M-H]$^-$ data and content of the phenolic compounds identified in the methanol extract obtained from *C. tabularis* leaves [a].

	Compounds[b]	Rt (min)	UV (nm)	Formula (M)	[M-H]$^-$, m/z	MS^2[M-H]$^-$, m/z (%)	Extract (mg/Kg $_{dry\ extract}$)[d]
Proanthocyanidins							
Catechin/epicatechin dimers 561							
1	Dimer 1	12.2	280	$C_{30}H_{26}O_{11}$	561.1402	409(60), 391(50), 289(100)	358.03 ± 31.00
3	Dimer 2	15.8	280	$C_{30}H_{26}O_{11}$	561.1401	409(100), 391(40), 289(50)	2 308.67 ± 184.84[b]
4	Dimer 3	16.1	280	$C_{30}H_{26}O_{11}$	561.1406	409(35), 391(50), 289(100)	221.81 ± 19.77
5	Dimer 4	16.8	280	$C_{30}H_{26}O_{11}$	561.1405	409(100), 391(35), 289(65)	109.18 ± 20.56
6	Dimer 5	17.2	280	$C_{30}H_{26}O_{11}$	561.1412	409(100), 391(55), 289(70)	223.00 ± 12.90
8	Dimer 6	18.8	280	$C_{30}H_{26}O_{11}$	561.1408	409(70), 391(60), 289(100)	173.09 ± 21.46
Catechin/epicatechin trimers 833							
7	Trimer 1	18.1	280	$C_{45}H_{38}O_{16}$	833.2090	681(50), 663(100), 561(90), 529(80), 409(40), 391(75), 289(50)	2 045.13 ± 100.94
9	Trimer 2	19.2	280	$C_{45}H_{38}O_{16}$	833.2092	681(30), 663(50), 561(100), 529(70), 409(35), 391(50), 289(20)	1 631.73 ± 55.02
10	Trimer 3	20.2	280	$C_{45}H_{38}O_{16}$	833.2089	681(45), 663(60), 561(100), 529(77), 409(40), 391(40), 289(35)	8 279.20 ± 229.65
11	Trimer 4	21.4	280	$C_{45}H_{38}O_{16}$	833.2093	681(40), 663(55), 561(55), 529(100), 409(45), 391(15), 289(15)	197.39 ± 31.07
12	Trimer 5	21.7	280	$C_{45}H_{38}O_{16}$	833.2089	681(45), 663(55), 561(100), 529(95), 409(35), 391(60), 289(40)	193.276 ± 25.69
15	Trimer 6	23.6	280	$C_{45}H_{38}O_{16}$	833.2092	681(45), 663(60), 561(100), 529(80), 409(40), 391(40), 289(30)	12 839.08 ± 570.51
Catechin/epicatechin trimers 817							
14	Trimer 7	22.9	---	$C_{45}H_{38}O_{15}$	817.2138	665(20), 647(20), 561(100), 529(35), 409(35), 391(40), 289(40)	153.77 ± 14.51
17	Trimer 8	25.8	280	$C_{45}H_{38}O_{15}$	817.2140	665(30), 647(20), 561(100), 529(20), 409(35), 391(50), 289(50)	217.69 ± 23.69
19	Trimer 9	26.5	---	$C_{45}H_{38}O_{15}$	817.2135	665(35), 647(15), 561(100), 529(10), 409(35), 391(50), 289(50)	120.63 ± 26.15
20	Trimer 10	27.1	---	$C_{45}H_{38}O_{15}$	817.2137	665(10), 647(20), 561(100), 529(45), 409(55), 391(25), 289(20)	125.63 ± 8.47
22	Trimer 11	28.3	280	$C_{45}H_{38}O_{15}$	817.2137	665(5), 647(20), 561(100), 529(45), 409(50), 391(20), 289(20)	599.08 ± 65.53
Flavonoids							
13	Kpf-Hx	22.5	267, 298sh, 348	$C_{21}H_{20}O_{11}$	447.0933	285(100)	580.92 ± 18.17
16	Qct-Rh	24.9	255,266sh, 288sh,350	$C_{21}H_{20}O_{11}$	447.0940	301(100)	10 021.22 ± 231.01
18	Lut-Hx	26.2	255sh, 268, 340	$C_{21}H_{20}O_{11}$	447.0937	285(100)	498.50 ± 15.43
21	diMeQct-Hx	27.8	250, 268sh, 296, 352	$C_{23}H_{24}O_{12}$	491.1195	476(100), 329(90)	152.22 ± 8.82
23	Kpf-Rh	28.8	266, 296sh, 348	$C_{21}H_{20}O_{10}$	431.0996	285(100)	63.43 ± 6.81
24	Isrh-Hx	29.0	-----[c]	$C_{22}H_{22}O_{12}$	477.1059	315(100)	67.18 ± 7.58
25	diMeQct-Hx	29.4	-----[c]	$C_{23}H_{24}O_{12}$	491.1197	329(100)	720.08 ± 39.06
							$\sum^d$ = 41 899.95 ± 1 768.87

[a] Main observed fragments. Other ions have been detected but were not significant. MS ions and their relative abundance were obtained by ESI(Ion Trap), except [M-H]$^-$, which was obtained by ESI-QTOF.
[b] Kpf: kaempferol; Qct: quercetin; Lut: luteolin; Isrh: isorhamnetin; diMeQct: diMethyl-quercetin; Rh: rhamnoside; Hx: hexoside.
[c] Coelutes with other compounds and its UV spectrum could not be well observed.
[d] Results correspond to mean ± SD (n = 3).

Effects on enzymatic targets

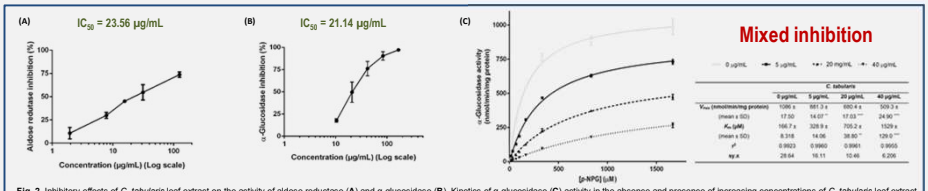

Fig. 2. Inhibitory effects of *C. tabularis* leaf extract on the activity of aldose reductase (**A**) and α-glucosidase (**B**). Kinetics of α-glucosidase (**C**) activity in the absence and presence of increasing concentrations of *C. tabularis* leaf extract. Results represent the mean ± SD of three independent experiments, performed in triplicate. Statistical significance: ** $p < 0.01$ and **** $p < 0.0001$ (ANOVA, Tukey's multiple comparison test).

➢ Neglectable α-amylase and pancreatic lipase inhibition

Effects on radical species

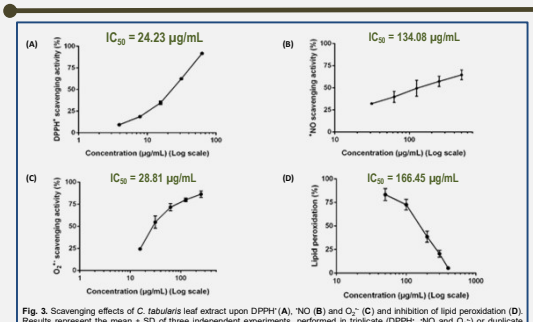

Fig. 3. Scavenging effects of *C. tabularis* leaf extract upon DPPH$^•$ (**A**), $^•$NO (**B**) and $O_2^{•-}$ (**C**) and inhibition of lipid peroxidation (**D**). Results represent the mean ± SD of three independent experiments, performed in triplicate (DPPH$^•$, $^•$NO and $O_2^{•-}$) or duplicate (lipid peroxidation).

Effects on inflammatory mediators

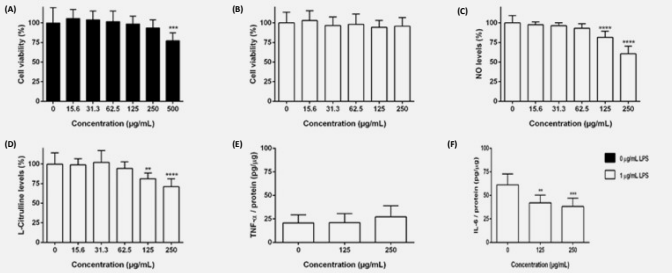

Fig. 4. Effects of *C. tabularis* leaf extract towards the cell viability of unstimulated (**A**) and LPS-stimulated (**B**) RAW 264.7 macrophages. Effects upon NO (**C**), L-citrulline (**D**), TNF-α (**E**) and IL-6 (**F**) levels in LPS-stimulated RAW 264.7 macrophages. Cells were pre-treated for 2 h with the extract, followed by 22 h co-treatment with LPS (1 µg/mL). Results represent the mean ± SD of at least four independent experiments, performed in triplicate. Statistical significance: * $p < 0.05$, ** $p < 0.01$ and **** $p < 0.0001$ compared to the respective control (no extract added) (ANOVA, Tukey's multiple comparison test).

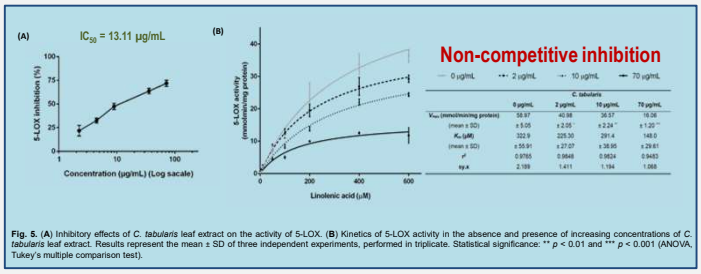

Fig. 5. (**A**) Inhibitory effects of *C. tabularis* leaf extract on the activity of 5-LOX. (**B**) Kinetics of 5-LOX activity in the absence and presence of increasing concentrations of *C. tabularis* leaf extract. Results represent the mean ± SD of three independent experiments, performed in triplicate. Statistical significance: ** $p < 0.01$ and *** $p < 0.001$ (ANOVA, Tukey's multiple comparison test).

CONCLUSIONS

Experimental data herein gathered evidence that *C. tabularis* leaves impact the enzymatic systems that are enrolled in the progression of metabolic dysregulations, particularly involving carbohydrate dysfunctions, potentially neutralizing also the radical storm observed on these conditions. Moreover, the extract obtained from *C. tabularis* leaf is suggested to mitigate LPS-induced inflammation in the selected cell model, and, consequently, might be considered for the lessening the chronic inflammatory condition enrolled to metabolic syndrome. Several phenolic constituents, including proanthocyanidins and flavonols, were here identified for the first time on the species and might be involved on the observed biological activities. Recorded results prompted us to deepen this phenolic-biologic relationship, additional experiments being currently carried out to further valorise the species and its active phytochemicals.

References:
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Acknowledgments
The work was supported through the project UIDB/50006/2020, funded by FCT/MCTES through national funds. C. Andrade thanks FCT (Fundação para a Ciência e Tecnologia) and ESF (European Social Fund) through POCH (Programa Operacional Capital Humano) for his/her PhD grant ref. SFRH/BD/131431/2017. N. Gomes is indebted to national funds through FCT, under the project CEECIND/03037/2017.