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Flavonoids extraction from Alpinia zerumbet (Pers.) Burtt et Smith leaves using different procedures
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MATERIAL AND METHODS

Materials

Samples of *Alpinia zerumbet* (Pers.) Burtt *et* Smith leaves were collected in Rio de Janeiro (Rio de Janeiro state, Brazil), in the "Núcleo de Pesquisas de Produtos Naturais", in the Universidade Federal do Rio de Janeiro. Voucher specimens were identified and are deposited at the Herbarium of Rio de Janeiro Botanical Garden, accession number RB 433485.

All chemicals used in analysis as methanol and phosphoric acid were of HPLC grades and were purchased from Merck. MilliQ water was utilized to HPLC mobile phase and sample preparation. Kaempferol-3-*O*-glucuronide was isolated from *Alpinia zerumbet* and identified by Nuclear Magnetic Resonance (NMR) [4]. Rutin was purchased from Merck®.

Preparation of extracts

Leaves of *A. zerumbet* were collected from adult plants, in the morning, then plant material was dried for 3 d in stove (60ºC) and macerated in 70% ethanol or distillated water, in the same proportion of 1 g dried leaves/20 mL solvent.

It was evaluated four extraction methods from dried leaves: maceration in shaker at 100 rpm, ultrasonic bath (40 kHz, Thornton Unique, model 1400 USC), microwave (PANASONIC® - Auto Sensor Diet, full power) and stirring (Table 1). In the microwave extraction, the suspensions were irradiated under microwaves in pre-setting procedures (3 s power on, 60 s off) for three times to the desired temperature about 60 and 70ºC. The temperature was measured after turn off the microwave using a thermometer into extracts in Becker. For the ultrasonic extraction, the 30 mL flask containing 1 g of dried leaves plus 20 mL of one of extracting solvents was partially immersed into the ultrasonic bath and temperature was controlled.

Table 1. Specifications of extraction methods.

Extraction methods	Extracting solvents	Temperaturea	Extraction time
Maceration	distillated water and 70% ethanol	25oC	3 d
Ultrasonic		40 and 60oC	45 min
Microwave		60 and 70oC	3x (3 s)
Stirring		50 and 60oC	60 min

[a]Data are related to water and ethanol 70%, respectively.

Crude extracts were filtered in vacuum through a Whattman® filter (110 mm Ø, 1). Aqueous extracts were frozen, liophylizated and the hydroalcoholic extracts were evaporated to dry under reduced pressure at 60ºC. The dried weight was measured. The yielding was defined as follows: (crude extract weight/plant material weight) x 100. The crude extract obtained by each extraction technique was analyzed directly by TLC and HPLC.

TLC analysis

Aliquots of standards and crude extracts were spotted on TLC plate (silica gel 60 $F_{254\ nm}$, Merck®) and developed in the mobile phase ethyl acetate:formic acid:water (65:20:15, v/v/v). Components were visualized under ultraviolet light (λ 254 and 366 nm, Model UVGL-58 Upland/EUA) and detected by spraying the TLC plates with reagent NP (2-aminoethyl-phenyl-borate, 1 $mg.L^{-1}$ in ethanol, Spectrum) and PEG (5% poliethyleneglycol-4000, Fluka®). The flavonoids standards

[a]Values indicate the averages of the three replicates.

Hydroalcoholic crude extracts analysis using HPLC revealed six main compounds, among them the peak corresponding to rutin (RT: 31.42 min) and kaempferol-3-*O*-glucuronide (RT: 34.49 min) can be observed right after 30 min, without interference of other components (Figure 1). These flavonoids were also verified through TLC technique.

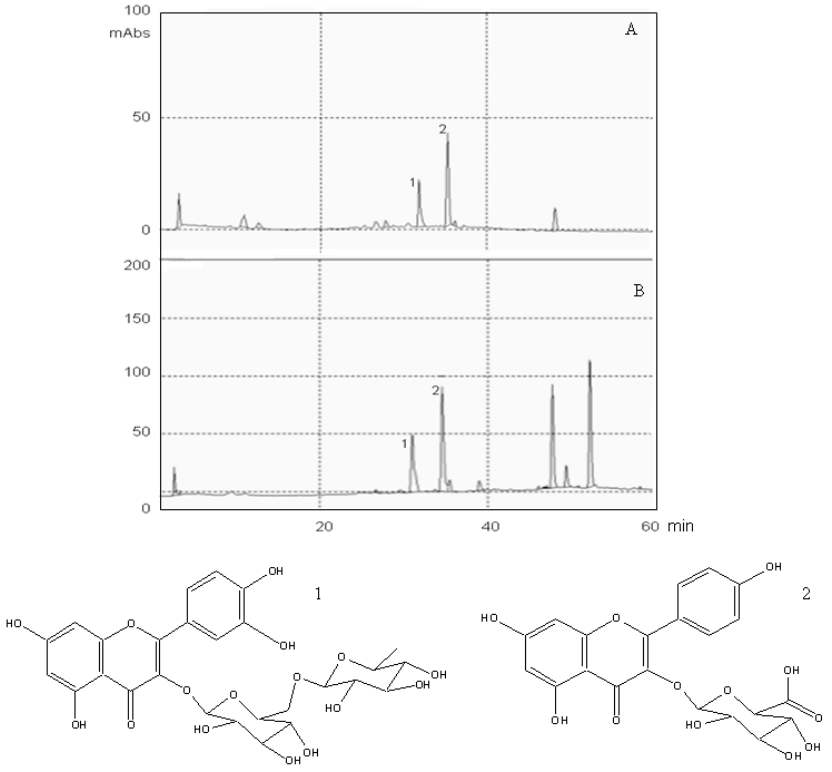

Figure 1. Chromatographic profiles (HPLC) of aqueous (**A**) and hydroalcoholic (**B**) extracts of *Alpinia zerumbet* obtained by ultrasonic extraction: rutin (**1**, RT 31.42 min) and kaempferol-3-*O*-glucuronide (**2**, RT 34.49 min) at 360 nm.

Chromatographic profiles of crude extracts obtained through different extraction methods and solvents were similar. The greater variation was achieved in relation to relative flavonoids content (Figure 2). The visualization of chromatographic profiles from different extraction technique and extracting solvent of each sample allowed to evaluate the qualitative and quantitative changes in secondary metabolite content and revealed the most appropriate system to obtain bioactive compounds from *A. zerumbet* leaves.

CONCLUSION

From the results obtained in the current study, the relative proportion of these flavonoids was reduced by maceration conventional technique, while microwave and ultrasonic techniques in combination with 70% ethanol solvent were the most efficient. It may suggest that microwave and ultrasonic methods using 70% ethanol are suitable for fast extraction of flavonoids in a simple way, also considering extraction yield and extraction time. These methods also permitted the acquisition of flavonoids from reduced raw plant material.

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Resumo: O presente estudo teve como objetivo verificar a melhor metodologia de extração para rápida e eficiente obtenção de flavonóides a partir de *Alpinia zerumbet*. Folhas secas foram extraídas com água destilada e etanol 70%, utilizando as metodologias de extração: maceração sob agitação, ultrassom, microondas e agitador. Para verificação dos flavonóides rutina e kaempferol-3-*O*-glicuronídeo foram utilizadas as técnicas de CCD e CLAE em fase reversa. O solvente etanol 70% foi mais eficiente como extrator. Para as metodologias ultrassom, microondas e agitador, não houve variação significativa para o rendimento utilizando etanol 70% (11 a 14%). A concentração relativa de rutina e kaempferol-3-*O*-glicuronídeo, respectivamente, foi maior pelos métodos de extração por ultrassom (1,5 e 5,62 mg g^{-1} folha seca) e microondas (1,0 e 6,64 mg g^{-1} folha seca), utilizando etanol 70%. Procedimentos rápidos e simplificados de extração otimizam o trabalho fitoquímico e a obtenção de metabólitos secundários.

Palavras-chave: cromatografia líquida de alta eficiência, maceração, microondas, ultrassom, Zingiberaceae

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