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# Chemical standardization of the aqueous extract of *Cecropia glaziovii* Sneth endowed with antihypertensive, bronchodilator, antiacid secretion and antidepressant-like activities

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## Abstract

This study reports the extraction process and standardization of the aqueous extract (AE) of a *Cecropia* species aiming its pharmacological characterization as a phytomedicine to be used in primary health care. The plant was originally collected in its environment, and was thereafter specially cultivated for the present work. To standardize the plant AE, several 2.0% tea of the dried leaves were prepared under controlled conditions and freeze dried. The AE (20% yield) was partitioned with *n*-butanol yielding the butanolic fraction (BuF; 1% yield). The activity of AE on vital organ functions (cardiovascular, respiratory, gastrointestinal and central nervous system) was determined in vivo. The effects of AE were compared to those of BuF in the same models and the relative potency determined. BuF was further evaluated in representative in vitro models to assess possible mechanisms of action. Chemical constituents of BuF were isolated in preparative HPLC columns yielding 10 highly purified compounds chemically identified as catechins (2), procyanidins (4), flavonoids (2), mixed sugars (1) and chlorogenic acid. All the compounds were identified by chemical analytic instrumentation ($^{13}$C-NMR, $^{1}$H-NMR, LC-MS). Their relative concentrations in AE were ca 12% catechins, 19% procyanidins and 19% flavonoids. The pharmacological activity of the standardized AE is reported in accompanying papers.


***Keywords:*** Medicinal plants; Standardization; Cecropia; Catechins; Flavonoids; Phytomedicine

## Introduction

Phytomedicines were used as remedies till the middle of the past century when they were progressively replaced by synthetic derivatives of better quality and easy control. Many of these products remained in use in folk medicine, although uncontrolled. In the search of new prototypes, the same products have been re-evaluated according to new standards of efficacy and safety.

*Cecropia glaziovii* Sneth is a tree characteristic of the Brazilian southeast coast, reputed for its medicinal properties like other species of the genus. This species was selected by the Brazilian Ministry of Health (CEME Program, 1984–1998) as a model to develop phytomedicines to be used in public health. The genus is well

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known in Brazilian folklore because the species houses the slot, and because they grow around devastated areas as a protection of the forest.

The Cecropiaceae family includes six genuses, the most important of them is the genus Cecropia formed by ca 75 tropical species usually found in lowlands, around dense forests, lakes and recently devastated areas. Many sea coastal species are dispersed in the Brazilian Atlantic forest; among them *C. glaziovii, C. hololeuca, C. leucocoma, C, lyratiloba, C. obtusa, C. pachystachya* and *C. scabra* (Angely, 1969; Magalhães, 2000).

Cecropia extracts were used in French medicine to treat heart failure (Bardet, 1908; Gilbert and Michel, 1920) and cough (Chernoviz, 1927); these indications were incorporated in Brazilian Pharmaceutical Formularies which extended the plant use to bronchitis, dyspnea and asthma (Coimbra, 1958). The syrup of *C. hololeuca* was included in the first Brazilian Pharmacopeia (Silva, 1929). A miscellany of other less probable activities, important to the first half of the past century, crowned the folk use to treat gonorrhea, bleeding, skin ulceration and diarrhea (Bahia, 1979).

Flavonoids, procyanidins, catechins and glycosides were isolated from the plant, but no specific pharmacological mechanisms were attributed to these compounds. Thus the molecular mechanisms involved in the plant activities were seldom reported (Lapa et al., 1999; Lacaille-Dubois et al., 2001).

For the present studies *C. glaziovii* Sneth was collected, cultivated and grown under controlled conditions (Magalhães, 2000). Following previous indication (Coimbra, 1958), the 2.0% water extract of the dried leaves was prepared and tested *per os* for pharmacological effects on rodent vital functions; semi-purified fractions were prepared, the effects were confirmed and the relative concentration of the chemical constituents was determined. After chemical standardization of the active crude extract, its toxicity after long-term administration was determined according to international protocols using rodents and dogs (World Health Organization, 1975; ICH, 2000). At the same time the mechanisms of action of the identified chemicals in the cardiovascular, respiratory, CNS and gastrointestinal functions were assessed using in vivo and in vitro models.

The plant extraction, chemical purification, isolation and identification of the purified constituents, as well as their relative concentrations in the aqueous extract (AE) are described in this paper.

## Materials and methods

*C. glaziovii* Sneth was cultivated in Campinas, State of São Paulo, Brazil for pharmacological and chemical studies (Magalhães, 2000). The leaves were dried under shade for 2 days complemented by a fast drying process at 40 °C. The dried leaves were easily cut and ground to 1 mm size before storage at room temperature in sealed plastic bags. The AE (2%) was prepared with hot distilled water (72 °C) during 30 min with 4–5 stirrings in the period. The AE was filtered, the total volume was measured and the extraction yield (20%) determined with the dry weight of 1 ml duplicate samples. The AE was concentrated under vacuum at 50 °C to a fifth of its volume before freeze drying. For the standardization procedures, the concentrated AE was partitioned with *n*-butanol ($3 \times 0.2$ l) and both the resulting aqueous fraction (AF) and *n*-butanol fraction (BuF) were freeze dried after evaporation of the organic solvent. The extraction yield in both BuF and AF was determined as described.

Chemical fingerprints of all 3 extracts were obtained using a Shimadzu HPLC (Japan). The system was constituted by 2 injector pumps (LC8A), a spectrophotometric detector UV–Vis (SPD 6AV) operating at 210 nm, SCL 8A controller, CR4A integrator and a FCV-100B fraction collector. Separation was performed on a Shimpack Prep-ODS ($25 \times 4.6$ cm) column, packed with round particles of 5 μm, eluted with a linear gradient of acetonitrile/deionized water (Nanopure System) from 10% to 20% in 45 min and acetonitrile 20% from 45 to 60 min with a constant flow rate of 10.0 ml/min. The resultant fractions were submitted to analytical HPLC monitored with a diode array detector to analyze coincident peaks. The yield of each substance was measured from the peak area relative to the total area of the chromatogram. All the fractions collected were identified in $^{13}$C-RNM, $^{1}$H-RNM and LC-MS.

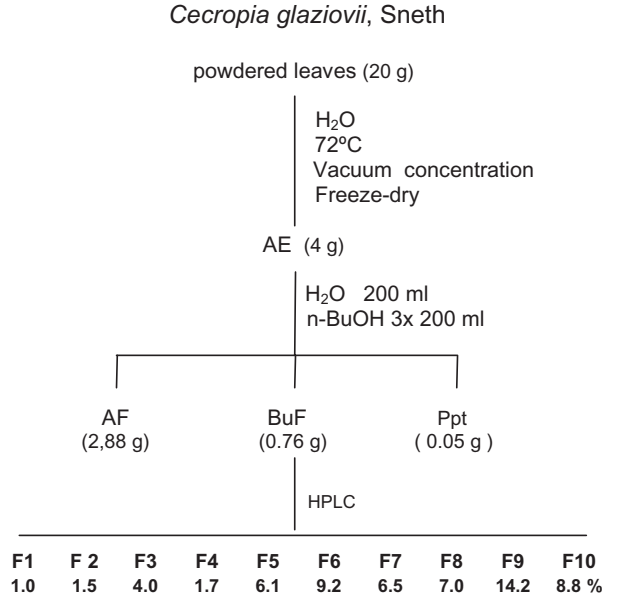


**Fig. 1.** Extraction procedure and yields of the different fractions and compounds isolated from *Cecropia glaziovii* Sneth.