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Purine alkaloids and phenolic compounds in three *Cola* species and *Garcinia kola* grown in Cameroon

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Abstract

Accessions of *Cola acuminata*, *Cola nitida* and *Cola anomala* were analysed based on the variation of polyphenol and alkaloid contents in order to gain insight on the genetic relationships within and between the taxonomic entities. These compounds were analysed at 280 nm by HPLC using a Photodiode Array Detector (PDA). Catechin, epicatechin, theobromine and caffeine were detected in *Cola* seeds. Among the three species, *C. nitida* is found to be highest in flavonoid and caffeine content while *C. anomala* possessed high amount of theobromine. Catechin was the dominant flavonoid. Caffeine was the major alkaloid in *Cola* seeds and was considered as one of the signature compounds due to its concentration range. The average concentrations in *C. acuminata*, *C. nitida* and *C. anomala* accessions were 11066±3166, 13761±2728 and 7013±2369 mg/kg FW respectively. *Garcinia kola* seeds displayed higher levels of total phenolic compounds compared to *Cola* sp. and lacked purine alkaloids and catechin. The former displayed many unidentified polyphenolic compounds most of which were apolar. Principal component and cluster analyses of *Cola* sp. categorized accessions into two groups. The first chemotype encompassed *C. anomala* accessions and exhibited high theobromine content. The second cluster enclosed *C. nitida* and *C. acuminata* accessions and displayed high caffeine and catechin contents. Genetic distances between the accessions varied from 0.0 to 1.26. The importance of *Cola* phenolic compounds and alkaloids in food chemistry and chemotaxonomy is discussed.

Keywords: Caffeine; Chemotype; *Cola* sp.; Dendrogram; *Garcinia kola*; Phenolic compounds; Theobromine

1. Introduction

Cola is a tropical African genus which belongs to the Sterculiaceae family. The genus comprises about 140 species and the most commonly consumed are *Cola acuminata* (Pal. de Beauv) Schott and Endl, *Cola nitida* (Vent) Schott and Endl and *Cola anomala* (Russel, 1956; Bodard, 1960; Dublin, 1965). Known as non-timber forest products, they are cultivated by subsistence farmers in association with cacao and/or coffee as a shade plant. Fruits consist of 1 to 10 follicles placed perpendicularly to the peduncle. The seeds, incorrectly called kolanuts, comprise two cotyledons (*C. nitida*) or 3–6 cotyledons (*C. acuminata* and *C. anomala*) (Fig. 1a). The plant was introduced to the Central and South American countries where it became popular during the Slave Trade of the 17th century. This popularity resulted from its reputation as a stimulant, increasing energy and strength, dispelling drowsiness and staving off hunger (Morton, 1992). These properties could be attributed to the richness of the seeds in purine alkaloids, polyphenols and sugar. *Cola* has a strong cultural significance in West Africa, where without these seeds, traditional hospitality, cultural and social ceremonies are considered incomplete. They are used by Moslems, who, according to their religion, cannot drink alcohol, but need a "social lubricant". In Europe, America and Nigeria, the seeds are used in the production of several pharmaceutical drugs, wines and liquors (Oladokun, 1982; Leung and Foster, 1996; Blancke, 2001).

Cola seeds are sometimes substituted by *Garcinia kola* Heckel (Guttifereae) seeds (Fig. 1b) which has a bitter astringent taste

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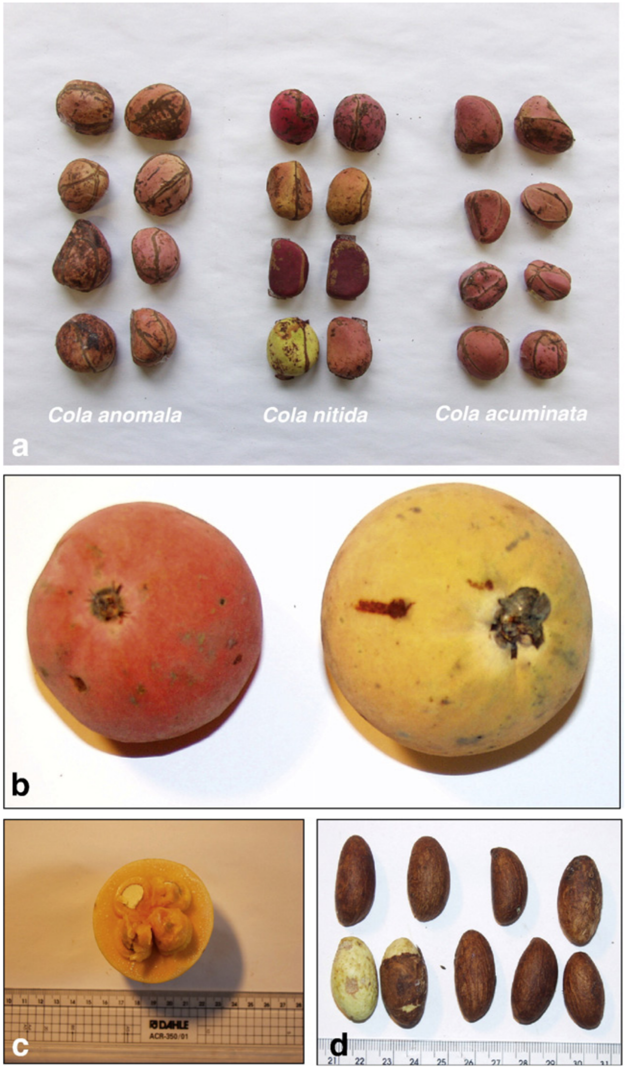

Fig. 1. Seeds of *Cola* sp. (a), fruits of *Garcinia kola* (b), transversal section of *G. kola* showing the disposition of seeds in the fruits (c) and seeds of *G. kola* (d).

resembling that of raw coffee seeds, followed by a slight sweetness. The bitter taste gained the seed its common name "bitter kola". The tree is used extensively in African traditional medicine for the treatment of various diseases (Farombi et al., 2002; Onunkwo et al., 2004). Phytochemical analyses of *G. kola* seeds have revealed that they contain a complex mixture of phenolic compounds including biflavonoids, xanthones, benzophenones and related triterpenes (Iwu et al., 1990; Waterman and Hussain, 1983). Biflavonoids known as kolaviron in *G. kola*, are the predominant compounds.

Flavonoids are widely distributed secondary plant metabolites which prevent degenerative diseases associated with oxidative stress (for review see Pourcel et al., 2006; Santos-Buelga and Scalbert, 2000). Alkaloids are low molecular weight nitrogen-containing substances with characteristic toxicity and pharmacological activity (Ashihara and Crozier, 1999, 2001).

Polyphenols have been used in the chemotaxonomy of various crops like *Gossipium hirsitum* L., *G. barbadense* L. (Tchiegang and Bourély, 1990), *Prunus domestica* (Groh et al., 1994), *Sorghum bicolor* L. (Dicko et al., 2002), *Galanthus caucasicus*, *Magnolia obovata*, *Cocculus laurifolius*, *Veratrum lobelianum* (Tsakadze et al., 2005) and *Theobroma cacao* L. (Niemenak et al., 2006). Data from several different plants which produce them suggest that their biosynthesis and accumulation involve a highly regulated process that includes cell-, tissue-, development- and environment-specific controls.

The occurrence and distribution of purine alkaloids have been well studied in three main taxa: *Coffea* (Charrier and Berthaud, 1975), *Camellia* (Zheng et al., 2002) and *Theobroma* (Hammerstone et al., 1994). Other purine alkaloids producing taxa like *Cola*, *Ilex* and *Paullinia* have been poorly investigated (Weckerle et al., 2003) most likely because of their weaker economic importance and/or lower species diversity. Since purine alkaloids are useful in chemosystematics (Hammerstone et al., 1994) and also have both ecological (Kretschmar and Baumann, 1999; Hollingsworth et al., 2002) and ethnobotanical (Beck, 1990) significance, our task was to evaluate one of the lesser known genera, the genus *Cola*.

The present study investigated the biochemical composition of three sociologically and ecologically important *Cola* species. Different ecotypes were considered in order to identify chemotypes for the establishment of a core collection and to generate dendrograms which would reflect species homologies. Exploitation of such traits would increase our knowledge of the genetic variability available. Quantitative and qualitative comparisons of the flavonoid and alkaloid contents of *Cola* sp. and *G. kola* were also undertaken.

2. Materials and methods

2.1. Plant material

Mature fresh seeds of sixteen accessions of *C. acuminata* ((Pal. De Beauvois) Schott and Endlicher), twelve of *C. nitida* ((Vent) Schott and Endlicher), twenty-one of *C. anomala* (Schott and Endlicher) and one accession of *G. kola* were harvested randomly from trees which were at least 10 years old from different sites in Cameroon (Table 1). Analysis of *G. kola* was done to find the similarity/dissimilarity with *Cola* sp. since they are customary commercialised together. The seeds were analysed at Biozentrum KleinFlottbek, University of Hamburg, Germany where upon arrival, they were stored at −20 °C.

2.2. Reagents and standards

Epicatechin was obtained from Sigma. Protocatechiuc acid and catechin were from Aldrich and Fluka while caffeine and theobromine were from Merck. All solvents used were of analytical grade purchased from Merck (Darmstadt, Germany). Water was purified by a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.3. Extraction of polyphenols

For total phenol extraction 500 mg of fresh *Cola* seed were ground in acetone/water 60:40 (v/v) using an Ultra-Turrax T25 (Janke & Kunkel IKA®) blender. The phenolic compounds were extracted by constantly shaking the blend samples on ice three