

Phenolic compounds in the kola nut (*Cola nitida* and *Cola acuminata*) (Sterculiaceae) in Africa

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Abstract: Phenolic compounds and total phenol in the two edible *Cola* species, *C. nitida* and *C. acuminata*, were analyzed by paper chromatography, using five solvent systems. N-butyl alcohol, acetic acid, water in the proportion of (4:1:5) yielded the highest number of phenolic compounds; catechin, quinic acid, tannic acid, and chlorogenic acid were determined to be the major components of the kola nut. There were marked differences in the amount of total phenol in each species and color characteristics-related differences within species. The percent total phenol in *C. nitida* was higher than in *C. acuminata*.

Key words: Chemical composition, phenolic compounds, biochemistry.

Phenolic compounds are widely distributed in the plant Kingdom and are important in fruits because they are responsible for the color and flavor of the fresh fruit and processed products (Lee and Jaworski 1987). They are particularly important products in enology and contribute to the organoleptic nature of wines (flavors, astringency, and hardness). They are also important in chemotaxonomy and plant pathology. Phenolic compounds have been implicated in host resistance to pathogens. Phenolic compounds of many fruits, such as apples, pears, and grapes have been analyzed (Hume 1953, Williams 1958, Ndubizu 1976, Lee and Jaworski 1987). The kola nut is a tropical tree crop which is important for its tonic and stimulating effect. To date, no attempt has been made to analyze its phenolic compounds. This study was conducted to elucidate the phenolic compounds of the kola nut and to ascertain any major difference in the phenolic compounds of the two Nigerian *Cola* species.

MATERIAL AND METHODS

Kola nuts were harvested from kola trees at the Cocoa Research Institute of Nigeria, Ibadan

Orchard. The nuts were removed from their pods, the testa (skin) removed by soaking in water for 24 hours.

Extraction and separation of phenolic compounds: Fifty grams of kola nuts were triturated in 90% methanol (Ndubizu 1976). For the determination of the total phenol, Rossi and Singleton's (1965) Folin - Ciocalteus reagent method was used, while separation of the phenolic compounds was done according to Ndubizu (1976). The extracts were chromatographed in descending order in the following solvents: butanol acetic acid water (BAW) 4:1:5 (upper layer). Forestal solution; chloroform acetic acid water; Benzene acetic acid water (6:1:3); Sodium chloride in 2N acetic acid. The chromatograms were air-dried, and viewed under ultraviolet light. The spots were marked and later developed with vanillin hydrochloride and 1% ferric chloride in .1N potassium ferrocyanide.

A two-dimensional chromatography in Butanol, acetic acid water (4:1:2) and 2% acetic acid was also carried out with a Whatman No. 1 chromatography paper. When viewed under ultraviolet light, four blue fluo-

rescent bands were seen. For confirmation of the spots obtained from chromatograms, 0.1% of pure phenolic compounds were spotted along with the ethyl acetate extract of kola nut and chromatographed in B.W.A. (4:1:5) on Whatman chromatography paper 1.

RESULTS AND DISCUSSION

The total phenol content of each species of kola nut is shown in Table 1. *C. nitida* has a higher phenolic content than *C. acuminata*. Total phenol varies across different species, being higher in the red nut than the white or pink varieties. These results clearly indicate the presence of phenolic constituents in large quantities in the kola nut as compared with fruits such as grapes, pears, peaches, and apples. According to Van-Buren (1970) the phenolics in these fruits range from 4.5 mg/100g; 2.4 mg/100 g to 0.45 mg/100g fresh wt. Of the two *Cola* species analyzed, *C. nitida* contained more total phenol. This supports the general opinion that *C. nitida* is more astringent than *C. acuminata*; because astringency is based on the phenol content in fruits. There also are intra-species differences in concentrations of total phenol. For example, in *C. nitida*, the quantity of total phenol in the red nut was three times that of white and pink nuts; but in *C. acuminata* the difference was not significant. The Rf values of different spots obtained from kola nut extracts, under different solvent systems shows that B.W.A. (4:1:5) had the resolution of phenolic compounds, with 6 spots, followed by forestal solution and benzene acetic acid water. Morakinyo (1978) obtained six spots from leaves of *C. nitida* and *C. acuminata* using B.A.W. and three spots in forestal solution.

The Rf's of the spots he obtained were 17, 25, 25, 41, 57, 65 and 75%, which were similar to the Rf's obtained in this study. Table 2 shows that catechin, epicatechin, chlorogenic acid, tannic acid, and quinic acid were identified from kola nut extracts as compared with authentic phenolic compounds when chromatographed with B.A.W. (4:1:5) on Whatman chromatography paper 1.

When standard reference samples were chromatographed along with kola nut extracts in B.A.W. (4:1:5) upper layer), spots with Rf's of .29, .44, .65, and .75 corresponded with quinic acid, chlorogenic acid, catechin, and epicatechin.

TABLE 1

Total phenol in two *Cola* species mg/100g fresh wt

| Color | <i>Cola nitida</i> | <i>Cola acuminata</i> |
|-------|--------------------|-----------------------|
| White | 4.45 | 3.37 |
| Pink | 6.12 | 4.17 |
| Red | 9.09 | — |

TABLE 2

Phenolic compounds identified from *Cola* extract using authentic phenolic compounds as reference, Solvent B.A.W. (4:1:5)

| Authentic phenolic compounds | <i>Cola nitida</i> | <i>Cola acuminata</i> |
|------------------------------|--------------------|-----------------------|
| Chlorogenic acid | ++ | ++ |
| Quinic acid | ++ | ++ |
| Tannic acid | +++ | +++ |
| Catechin | +++ | +++ |
| Epicatechin | +++ | +++ |
| Genitistic acid | — | — |
| Rubutin | — | — |

++ = Present
+++ = Present in large quantities
— = None

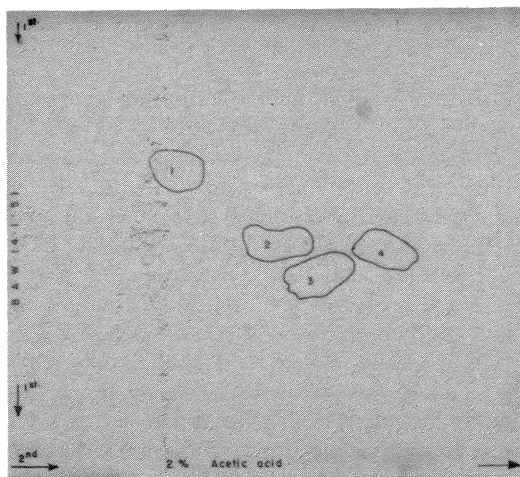


Fig. 1. Chromatogram of phenols of Kola nut extracted with methyl alcohol and developed first in B.A.W. and then in 2% acetic acid. Spot 1 is chlorogenic acid, spot 2 is tannic acid and spots 3 and 4 are the catechine. The chromatograms were sprayed with 3% Ferric chloride plus potassium ferrocyanide.

Chlorogenic acid is sometimes the predominant single phenolic compound in fruits. Its concentration can be as high as 0.25% in *Prunus* (Swain and Hills 1953) and is found in high concentrations in apples, which allows for its isolation and characterization (Hume 1953). Chlorogenic acid is the most important cinnamic acid derivative found in fruit (Hume 1953). In cocoa, which is related to kola, chlorogenic acid was found only in the young leaf and sap wood (Williams 1971).

Catechin and epicatechin, found in large quantities in kola nuts, are the most common forms of flavans (Herrman 1959, Harborne 1964, Lee and Jaworski 1987). They are present in particularly large quantities in higher plants. In cocoa, a total of six catechin-like substances have been reported mainly in the cotyledons with epicatechin as the main component (Harborne 1964). The results of this study agree with Harborne (1964). As stated above, catechin and epicatechin constitute the bulk of kola nut phenolics.

RESUMEN

Se analizaron los compuestos fenólicos y el fenol total de dos especies comestibles de *Cola nitida* y *C. acuminata*, mediante cromatografía en papel, y utilizando cinco sistemas de solventes. Los solventes que produjeron la más alta cantidad de compuestos fenólicos son alcohol butílico N, ácido acético y agua en la proporción respectiva de 4:1:5. Según se determinó, las siguientes sustancias constituyeron los componentes predominantes del fruto de la *Cola*: catequín, ácido quínico, ácido tánico y ácido clorogénico. Se evidenciaron diferencias notables en la cantidad total de fenol según especie y según las características relacionadas con el color del fruto. El porcentaje total de fenol en *C. nitida* superó al de *C. acuminata*.

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