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The Importance of Phytochemistry in the Taxonomy of Twenty-One Cola Species in Nigeria

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Abstract
Cola is a genus with about 125 species and the name was given by Schott and Endl 1832. They are mostly restricted to the forest regions of west Africa and 31 of which are found in Nigeria. They were classified mainly based on the floral types.Most of the Cola species are in the wild while few edible species are being cultivated like Cola nitida, Cola acuminata, Cola verticillate andColaanomala. These species are very difficult to separate from one another except by the number of cotyledons.The Cola leaves were collected from the field and dried in the laboratory. The leaves were tested using the standard methods of Sofowora (1993), Evans (2002) and Trease and Evans (1989). The leaves were tested for the presence or absence of Alkaloids, Saponins, Tannins, Anthraquinone Derivatives, Flavonoids, Terpenes, Balsams and Resins. Alkaloid was absent in all the Cola species because caffeine which is the alkaloid in Cola does not form precipitate with Mayers, Hager's and Dragendersreagents. Anthraquinone was only found in Cola nitida. Some of the Cola species have same chemical constituent.Most of the Cola species have quite a number of the chemical constituents indicating their close affinities. The differences noticed among the cola species in the number and types of chemical constituents can also be used to identify some of the taxa.

Key words: *Phytochemistry. Cola, Taxonomy, Nigeria.*

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I. Introduction

The genus Cola was given by Schott and Endl in 1832. It was separated from sterculia Linn on the bases of flower and fruit characters (Russel, 1955). Cola is indigenous to Africa (Airy-Shaw, 1985). Members are trees which grow in moist environment (Nyananyo, 2006). Some species of Cola are grown extensively in Africa, west indies, tropical areas of south America (Park, 1987).

Several attempts have been made in the past to describe the taxonomic position of Colaand its subdivisions (Schumann, 1900; Chevalier & Perrot, 1911; Bodard, 1962).

According to Russel (1955), the systematics of Cola species was in the state of "indescribable confusion" by the beginning of the twentieth century as a result of profusion of new species, named on the basis of very meager evidences. This confusion was cleared by two French Botanists, Augustus Chevalier and Emile Perrot in 1911.

Work on Cola have been concentrated on Cola nitida and acuminata. Accessions of Cola acuminata, C. nitida and C. anomala have been analyzed based on the variations of polyphenol and alkaloid contents in order to gain insight on the genetic relationships within and between the taxonomic entities (Niemenk et al., 2008). Sonibere et al., (2009) showed that the presence of alkaloids, saponins, tannin and cardenolides in C. acuminata, C. millenii, C. nitida and C. gigantea indicated their closeness taxonomically.

Chemical data are useful in all levels of the taxonomic hierarchy, the exact levels depends upon the particular compounds employed. As a general rule, the micromolecular data are most useful at the lower levels. Flavonoid are clearly useful at the lowers (Young, 1979; Doyle, 1983; Bain and Denford, 1985 and Park, 1987). The micromolecular approach to plant classification, though useful at generic and lower levels, is of very limited value at the higher taxonomic levels. Chemical data are being used either by recording the presence or absence of various compounds in different taxa or by comparing structural features and biosynthetic pathways of a common compound or chemically related compounds (Takhtajan, 1973).

II. Materials and Methods

Fresh Cola leaves from the field were air dried in the herbarium laboratory for three weeks. Leaveswere ground into powder using a Nakai HR-2818 blender. Standard methods of Sofowara (1982) and Trease and Evans (1989) were used for the screening.

1. Extraction

The powdered samples were macerated in redistilled mentholated spirit. Each of the extracts was suction-filled repeatedly till all the soluble compounds have been extracted which was indicated by the filtrate becoming clear. Each extract was evaporated to dryness in vacuum at 45^0C. These were used to test for alkaloids, tannins and anthraquinones

Test for Alkaloid

About 0.5g of each extract was stirred with 5ml of 1% aquoes hydrochloric acid in water bath. 1ml of the filtrate was treated with a few drops of Mayers reagent. Turbidity or precipitation with either of these reagents indicated the presence of alkaloids (Evans, 2002).

Test for Saponins (Froth Test)

A small quantity of the powdered samples was put in a test tube and 95% ethanol was added and boiled in a water bath. The content was filtered and 2.5ml of the filtrate was added to 10ml of distilled water in a test tube, shaken vigorously for about 30 seconds and allowed to stand for more than half an hour. The appearance of honey comb froth indicated the presence of saponins (Sofowora, 1993).

Test for Tannins

About 5g of each extract was stirred with 10ml of distilled water and then filtered. Few drops of 10% ferric chloride was added to the filtrate. Blue-black, green or blue-green precipitate indicate the presence of tannins (Evans, 2002).

Test for Anthraquinone Derivatives

About 5g of each plant extract was shaken with 10ml of benzene in a test tube. That was filtered and 5ml of 10% ammonia solution was added to the filtrate. The mixture was shaken. The appearance of a pink, red or violet colour in the lower portion of the ammonia showed the presence of free hydroxyl-anthraquinone (Evans, 2002).

Test for Flavonoids (Sodium Hydroxide Test)

About 5g of each of the powdered samples was detanned with acetone. The residue was extracted in warm water after evaporating the acetone in a water bath.

The mixture was filtered and the filtrate used for flavonoids and phenol tests. To 5ml of the detanned water extract was added 10% lead acetate solution. A yellow precipitate showed the presence of flavonoids (Segelman et al., 1971).

Test for Terpenes

About 5g of each of the powdered samples was extracted by maceration with 50ml of 95% ethyl acetate, filtered and the filtrate evaporated to dryness. The residue was dissolved in 10ml of anhydrous chloroform and then filtered. The filtrate was divided into two equal portions for sterol and terpenes tests. The first portion of the chloroform solution was mixed with 1ml acetic anhydride, followed by the addition of 1ml of concentrated sulphuric acid down the wall of the test tube to form a layer underneath. The formation of the reddish violet colour indicate the presence of terpenes (Sofowora, 1993).

Test for Balsams

Two drops of alcoholic ferric chloride solution was added to 5ml of 90% ethanol extract of the powdered samples. A dark green colour indicated the presence of balsams (Evans, 2002).

Test for Resins

15ml of petroleum ether extract was made from 0.1g of each of the powdered sample and filtered into a test tube. An equal volume of copper acetate solution was added and shaken vigorously and allowed to separate. Green coloration indicated the presence of resins. (Evans, 2002).

Test for Sterols (Salkowski's Test)

The second portion of the solution used for the test of terpenes was mixed with 2ml of concentrated carefully so that the sulphuric acid formed a layer. A reddish brown colour was form indicating the presence of a steroidal ring (Sofowora, 1993).

III. Results

The twenty-one (21) Cola species were screened for the following constituents; anthraquinone derivatives, saponins, terpenes, sterols, flavonoids, resins, balsams, alkaloids and tannins as presented in the table below.

Alkaloids were absent as shown in the table because caffein which is the alkaloid in Cola does not form precipitate with Mayers, Wagners, Hagers and Dragendrorffs reagents. Terpenes and sterols were present in most of the Cola species except *Colahispida, Colamegalphylla*and*Colamasupium.*

Anthraquinone was absent in all the taxa except in *Colanitiida*. Tannins were observed in *Cola rostrata* and*Cola gigantea*. Balsams were only detected in *C. anomala, C. nitida, C. rostrata, C. laurifolia* and *C.chlamydantha*. as indicated in the table below.

The presence of saponin was recorded in *C.acuminata, C.pachycarpa, C. lepidota, C. flaviflora, C. anomala, C. laurifolia* and *C. marsupium* as presented on the table below.

Flavonoids were present in *C. auminata, C. pachycarpa, C. lepidota, C. facifolia, C. flaviflora, C. lateritia, C. megalophylla, C. chlamydontha*and *C. marsupium.* Resins were found in all the species except *C. acuminata, C. millenii, C. lepidota, C. verticilata, C. lateritia, C. digitata, C. megalophylla, C. chlamydantha*and *C. marsupium.*

PHYTOCHEMICAL CHARACTERS OF SEVENTEEN COLA SPECIES

Taxa	Anthraquinone derivatives	Saponin	Terpenes	Sterol	Flavonoids	Resins	Balsams	Alkaloids	Tannins
C. glabra	-	-	+	+	+	+	-	-	-
C. acuminata	-	+	+	+	-	-	-	-	-
C. pachycarpa	-	-	+	+	+	+	-	-	-
C. hispida	-	-	-	-	+	+	-	-	-
C. millenii	-	+	+	+	-	-	-	-	-
C. lepidota	-	+	+	+	-	-	-	-	-
C. ficifolia	-	-	+	+	+	+	-	-	-
C. verticilata	-	+	+	+	-	-	-	-	-
C. flaviflora	-	-	+	+	-	+	-	-	-
C. lateritia	-	+	+	+	+	-	-	-	-
C. nigerica	-	-	+	+	+	+	-	-	-
C. anomala	-	-	+	+	+	+	+	-	-
C. digitata	-	-	+	+	+	-	-	-	-
C. heterophylla	-	-	+	+	+	+	-	-	-
C. nitida	+	-	+	+	+	+	+	-	-
C. rostrata	-	-	+	+	+	+	+	-	+
C. megalophylla	-	-	-	-	-	-	-	-	-
C. gigantea	-	-	+	+	+	-	-	-	+
C. laurifolia	-	+	+	+	+	+	+	-	-
C. chlamydantha	-	-	+	+	-	-	+	-	-
C. marsupium	-	+	-	-	-	-	-	-	-

+ = Present

- = Absent

IV. **Discussion**

The results of the investigations into the chemical constituents of the twenty-oneCola species revealed that they can be of good taxonomic importance. Although some of the Cola species have the same chemical constituents but some have distinctive elements that can clearly distinguish them from others.

Cola nitida which cannot be clearly separated from *Cola acuminata, Colaanomala*and*Colaverticillata* morphologically or on the field is the only species that showed the presence of anthraquinone.

Cola gigantea and*Colarostrota* showed close affinity to each other but the former has resins and balsam which are absent in the later.

Most of the Cola species contain terpenes, sterols and flavonoids which is an indication that the taxa have close affinities to each other. The blEndlng characters of the Cola species is a reason for them to be under

the same genus. *Cola marsupium* can easily be identified because it is the only taxon that showed the presence of saponin. Some species like *Colaheterophila, Cola glabra* and*Colanigerica*portray very close affinity to each other because they have terpenes, sterols, flavonoids and resins as their chemical constituents and therefore are very close. Some of the Cola species that can be grouped together because of possession of the same elements are *Cola gigantea, Cola digitata, Cola verticillate* and*Colamellenii* which have terpenes, sterol and flavonoids.

Although these Cola species may not be very close to each other as exhibited by their elemental possession, but when some other characters are being researched, they may form cluster. Therefore, chemical constituents alone cannot be a reason for whether a particular species should be included or excluded from the genus Cola but it gives an insight for the classification.

V. Conclusion

The variation in the phytochemical characters of the Cola species means that they are good diagnostic features. However, the similarities exhibited by some of the species are being used for different purposes more especially medicinally and to that effect the phytochemical screening of a particular species can be of great assistance to avoid either adulteration or its misconception.

Furthermore, the different chemical constituents are useful in the identification of the taxa when combined with other characters.

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