

Full Length Research Paper

Cultivars of *Codiaeum variegatum* (L.) Blume (Euphorbiaceae) show variability in phytochemical and cytological characteristics

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In the course of their evolution, plant developed chemical defences when attacked. These phytochemicals inadvertently protect humans against pathogens as antimicrobial medicines. Thus, six clone cultivars of *Codiaeum variegatum* (Spirale, Royal, Broad Spotted Guinea, Punctatum, Sunray and Royal-like) were chemically and cytologically investigated to evaluate their therapeutic potentials, values and variability. The shoots were relatively rich in alkaloids, cardiac glycosides, saponins, tannins, cardenolides, steroids, and phyllates. Flavonoids, phlobatannins, phenols and anthraquinones were sparingly present. Anthraquinones and cardenolides were absent in 'Spirale' while 'Sunray' lacked tannins, phlobatannins and phenols. Terpenes were found only in 'Sunray'. Overall, alkaloids were the most abundant with the highest mean value of 1.46% in 'Royal' while flavonoids and anthraquinones were trace, 0.002 and 0.003%, in 'Sunray' and 'Royal-like'. Five pigments, carotene (yellow-orange), phaeophytin (grey), chlorophyll a (blue-green) and b (green), and xanthophyll (yellow) were determined. 'Spirale', 'Royal', 'Broad Spotted Guinea' and 'Punctatum' had diploid chromosome number of $2n = 60$ while 'Sunray' and 'Royal-like' possessed $2n = 30$ and 24 respectively. 'Spirale' and 'Sunray' appeared to have reasonably diverged from the rest. Genetic mutation and chromosomes variability could account for the wide morphological variation and morphotypes in this plant species.

Key words: *Codiaeum variegatum*, cultivars, Euphorbiaceae, chromosome number, phytochemical, pigment.

INTRODUCTION

Plants have chemical defences against herbivores, man and other organisms in their community. However, many of these phytochemicals (e.g. stimulants, alkaloids, spices etc.) produced as exudates or latex when plants are bruised, cut or damaged have proved useful to humans as antibiotic or antifungal medicine (Audesirk et al., 2006; Simpson and Ogorzaly, 2000). The garden Crotons is an example of the most common plants in this group that have not been fully explored. The garden Crotons, *Codiaeum variegatum* (L.) Blume, are a group of beautifully variegated leafy perennial, tropical ornamental

herbs, shrubs or trees with glabrous branches and prominent leaf scars (Dutta, 2004). *Codiaeum* is the second largest genera (c. 700 species) of the family Euphorbiaceae which has about 7000 species. There are only six known basic species of *Codiaeum* (Taylor, 1938) from which, all other cultivars arose as mutants or hybrids.

They have glossy leathery leaves that are variable in shape, colour and variegation. The leaves are alternate, non-serrated but sometimes lobed. The shape varies from linear-lanceolate, oblong, elliptic, lanceolate, ovate, spatulate, fiddle-shaped to broad and obovate. Sometimes the leaf blade is interrupted along the midrib and become divided into an upper and lower part. The leaf is probably green in its original natural state, but in cultivated forms, it is variously marked, streaked, blotched or banded with green, white, the reds (orange, purple, pink, indigo, violet), yellow, crimson, scarlet, brown or cream colour when grown in appropriate light conditions

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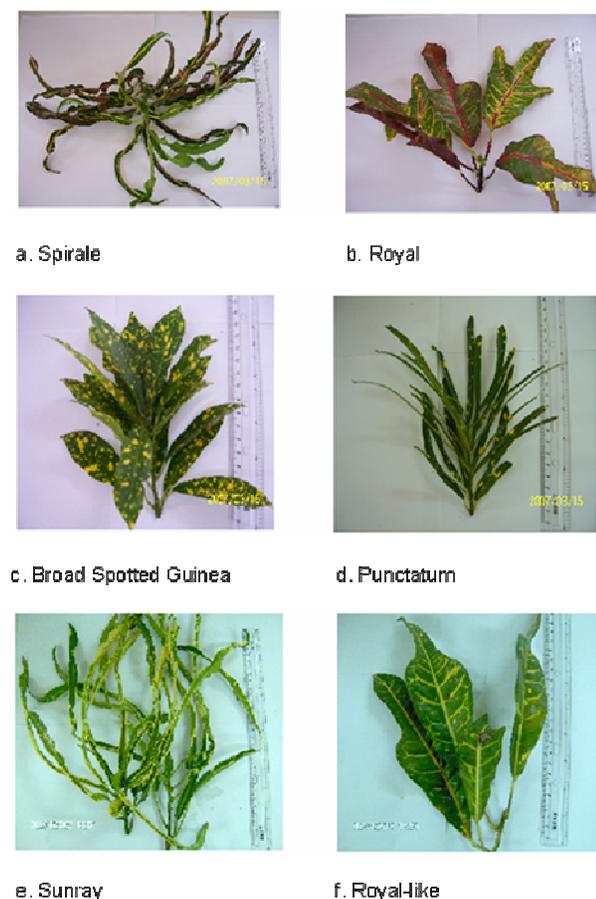


Figure 1. Cultivars of *Codiaeum variegatum*.

(Anon, 2003). The plant may change colour as it matures.

Apart from being a very popular ornamental in Nigeria, our team has been fascinated to this plant for its radiant color combination, strong mutation tendencies, resilience to fairly harsh weather, ease of propagation and diverse leaf shapes. At present, 56 cultivars are being maintained in the Biological garden of the Babcock University Germplasm Repository (BUGR), Ilishan-Remo. The beauty of the garden crotons is manifested when about 13 different colour combinations are featured in a flush of 10 - 26 leaves, such that no two leaves have identical patterns.

Garden crotons are natives of Malaccan Islands, between the Philippines and New Guinea. Its native habitats include India, Philippines, Sri Lanka, Thailand, Indonesia, Malaysia and some other Pacific Islands. They are also popular in East Asia and Java, Australia and now world over in the tropics (Stamps and Osborne, 2003; Taylor, 1938).

The latex produced from the bark, root and leaves are poisonous. It contains the toxin 5-deoxyingenol. The bark and roots can cause burns of the mouth while the latex

causes eczema in gardeners after repeated exposure. The exudates cause irritation to the skin and are used as purgative by humans and in domestic animals (Bronson, 2005).

Flowers of the garden croton plants are racemose, monoecious and usually unisexual. The male and female flowers are borne on different stalks and mature at different times. Most garden croton cultivars produce both male and female inflorescence from the same axial but most times the female inflorescence is developed earlier and fruits are formed before the males are produced (Esan et al., 2005).

Aside its ornamental value, it is also used for medicinal purposes. Root decoction is taken to treat gastric ulcers. Its leaves contain antibacterial and antiamebic properties (Moundipa et al., 2005). Freeze-dried leaf decoction of *C. variegatum* is taken as tea by Filipinos (Gertrudes, 2006). Drinking of crushed leaves cures diarrhoea. Young leaves of *Codiaeum*, *Pandanus macroieacceretia* (white part), coconut milk and sap of the root of *Areca catechu* is drunk for gonorrhoea treatment. Also, sap of the leaves is pressed out and mixed with coconut milk and spread over affected area of syphilis (Robert et al., 1988).

Phytochemicals are known to have protective and disease preventive properties. They also provide medicine for cell health and repairs, and inhibit cancerous-producing substances. They act as antioxidants against metabolite free radicals whose reactions damage the DNA and other cells constituents (Balch and Balch, 2000). The phytochemical screening of plant materials to determine the presence of bioactive constituents is, thus, vital in the knowledge of therapeutic properties of plants. Such bioactive chemical constituents analyzed in this study include alkaloids, anthraquinones, flavanoids, terpenes, steroid, phenol, saponins, tannins, phlobatanin and cardenolide. We also investigated the leaf colour bands and pigments, and the somatic chromosomes to deduce the basis of the wide morphological variability.

MATERIAL AND METHODS

Collection of samples

Fresh shoot samples of the six cultivars of *C. variegatum* (Figure 1) were collected from the Babcock University Germplasm Repository (BUGR). Identification was carried out from the compiled checklist maintained on the stock and verified by Systematists in the Department of Basic and Applied Sciences, Faculty of Science and Technology, Babcock University, Nigeria.

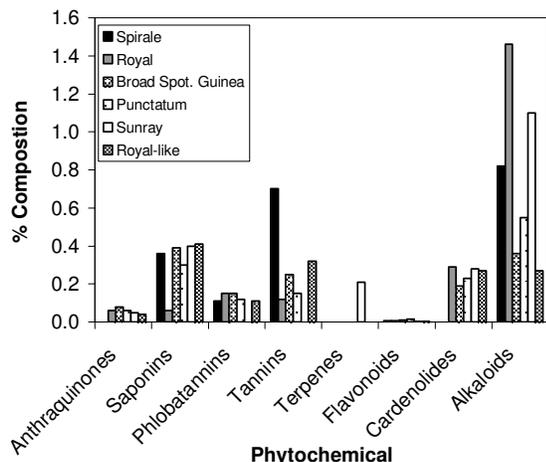
Phytochemical screening

Two-month room temperature air-dried samples of six cultivars of *C. variegatum* were blended and milled into fine powder. 10 g of the powdered sample was soaked in sealed conical flask containing 100 ml absolute ethanol for 48 h, filtered in Whatman filter Paper No. 1 and concentrated in water bath at 80°C. Pytochemical tests were carried out on ethanol extract of powdered dried sample using standard methods of Swain (1966), Harborne (1973), Sofowora (1993) among others.

Table 1. Qualitative analysis of phytochemicals of six cultivars of *Codiaeum variegatum*.

Phytochemical	Cultivars					
	Spirale	Royal	Broad Spotted Guinea	Punctatum	Sunray	Royal-like
Alkaloids	+++	+++	++	++	+++	++
Anthraquinones	-	+	+	+	+	+
Cardiac glycosides	+++	+++	+++	+++	+++	+++
Saponins	++	+	++	++	++	++
Phlobatannins	+	+	+	+	-	+
Tannins	+++	+	++	+	-	++
Cardenolides	-	++	++	++	++	++
Steroids	+++	+++	+++	+++	+++	+++
Terpenes	-	-	-	-	+	-
Flavonoids	+	+	+	+	+	+
Phenols	+	+	++	+	-	+
Phyllates/Phytic acids	+++	+++	+++	+++	+++	+++

+++ = Abundant; ++ = moderate; + = trace; - = absent.

**Figure 2.** Phytochemical composition of cultivars of *Codiaeum variegatum*.

Phytopigmentation

1 g of fresh and dried samples was weighed and ground in a mortar with about 1 ml acetone and further with 8 ml acetone. This was allowed to settle and decanted using Pasteur pipette into labeled test tubes. 2 ml of hexane was poured into the standing mixture and shaken properly. This was left to stand and two separate columns were formed. The upper column, which retained the pigments, were spotted on thin layer chromatography (TLC) plates (20 x 10 cm) which had been ruled out 1 cm at the top and bottom end. The bottom line serves as the imaginary start line and the top line serves as the solvent front. Each extract was spotted 1.5 cm apart with the aid of capillary tubes on the bottom line. This was allowed to develop in a separating jar containing a mixture of 3:1 hexane-acetone.

Cytology

Fresh stem stalks of six cultivars of *C. variegatum* were harvested from BUGR, dipped in water troughs and allowed to root. Ten-day

young roots were harvested at 2 h intervals for cytological analysis as described by Ogunwenmo (1999, 2005). Well spread somatic chromosomes were viewed at x1000 oil immersion and photographed with a National DC3-163 digital microscope with Motic Images 2000 1.3 digital capture camera.

RESULTS AND DISCUSSION

The shoots of *C. variegatum* were rich in alkaloids, cardiac glycosides, saponins, cardenolides, steroids, and phyllates/phytic acids. Phenol and its hydroxylated derivatives, flavonoids, occurred in trace amounts (0.002 - 0.02%) in most of the cultivars to non-existent in 'Sunray'. However, the hydrolyzable polyphenols, tannins, and its condensed form, phlobatannin, were present in reasonable amounts, 0.11 - 0.7%, aside 'Sunray'. Anthraquinones were sparingly present (0.04 - 0.08%) in the cultivars but absent in 'Spirale' in addition to cardenolides. Only 'Sunray' possessed terpenes. Overall, alkaloids were the most abundant with the highest mean value of 1.46% in 'Royal' while flavonoids and anthraquinones were least, 0.002 and 0.003%, in 'Sunray' and 'Royal-like' (Table 1 and Figure 2).

Thin layer chromatography of the fresh and dry samples showed that the plant contained five colour bands with corresponding Rf of five pigments: carotene (yellow-orange), phaeophytin (grey), chlorophyll a (blue-green), xanthophylls (yellow) and chlorophyll b (green). However, phaeophytin was absent from the fresh leaf sample of 'Sunray'. The Rf of fresh leaf samples were often more constant and closer to the standard (Table 2).

Cultivars 'Spirale', 'Royal', 'Broad Spotted Guinea' and 'Punctatum' had diploid chromosome number of $2n = 60$ while 'Sunray' and 'Royal-like' possessed $2n = 30$ and 24 respectively (Figure 3).

Alkaloids, cardiac glycosides, cardenolides, saponins, steroids, tannins, and phyllates/phytic acids occurred in

Table 2. Thin Layer chromatographic data for *Codiaeum variegatum* cultivars.

Photo-pigment	Band	R _f												
		*Std	Spirale		Royal		Broad Spotted Guinea		Punctatum		Sunray		Royal-like	
			fresh	dry	fresh	dry	fresh	dry	fresh	dry	fresh	dry	fresh	dry
Carotene	Yellow-orange	0.96	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99
Phaeophytin	Grey	0.70	0.70	0.86	0.70	0.86	0.70	0.86	0.70	0.86	0.52	0.86	0.70	0.86
Chlorophyll a	Blue-green	0.58	0.52	0.66	0.52	0.66	0.52	0.66	0.52	0.66	0.49	0.66	0.52	0.66
Xanthophyll	Yellow	0.44	0.49	0.62	0.49	0.62	0.49	0.62	0.49	0.62	0.36	0.62	0.49	0.62
Chlorophyll b	Green	0.48	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	-	0.36	0.36	0.36

* Tomkins and Miller (1994).

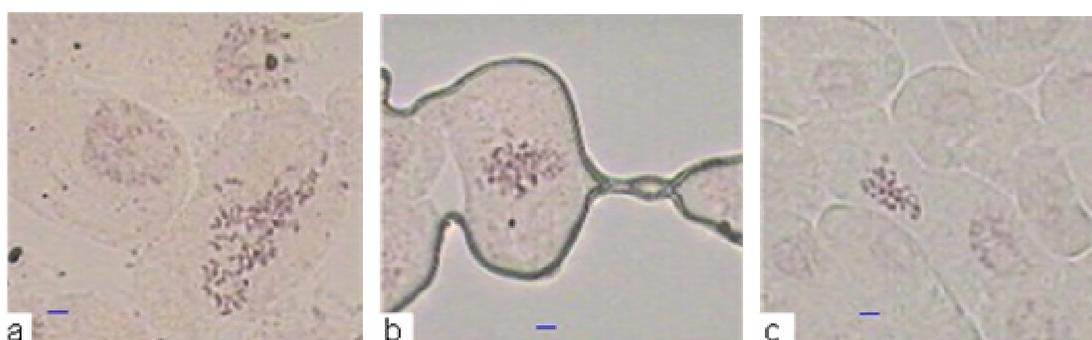


Figure 3. Somatic metaphase chromosome of *Codiaeum variegatum*. a. Spirale 2n = 60; b. Sunray 2n = 30; c. Royal-like 2n = 24; a-c. Scale bar = 10 μ m.

reasonable quantities in most of the cultivars. This may be related to their use for treatment of diarrhoea, gonorrhoea, syphilis and their antibacterial and antiamoebic activities. Useful alkaloids such as isopteropodine, pteropopine, isomitraphylline which help the white blood cells dispose harmful microorganisms and cell debris, and rynchophylline which improve cardiac conditions by reducing blood pressure, increasing circulation and inhibiting the accumulation of arteriosclerosis plaque and blood clots have been reported (Jeffery and Harborne, 2000). Steroids occur as glycosides which are important starting materials for synthesis of drugs. Plant glycosides have genins with 23 (cardenolides) or 24 (bufadienolides) carbon atoms synthesized from plants' C₂₁ steroids. Pure cardiac glycosides administered as injections or tablets inhibit active transport of K⁺ and Na⁺ through membranes thereby strengthening heart muscle and the power of systolic concentration against congestive heart failure (Clifford et al., 1973). The bitter taste of glycosides prevents birds and insects from eating immature fruits and seeds and also abates the decay of damaged plant tissues.

Hydroxylated phenols are toxic to microorganisms. Their relative activity increases with increasing level of oxidation (Scalbert, 1991) and, number of hydroxyl groups and their specific site (Geissman, 1963; Pashin,

et al., 1986). Phenols and flavonoid have the ability to complex with nucleophilic amino acids in proteins and the bacterial cell walls leading to enzyme inactivation and loss of function (Mason and Wasserman, 1987). Phenols accumulate macromolecules (cobalt, manganese and iron chromium, copper, etc.) some of which are necessary co-factors and activators of many enzymes responsible for phenol production and metabolism (Lovkova et al., 1999). Therefore, loss of enzymes for phenol and tannin production in 'Sunray' may result from genetic mutation or continued artificial selection for ornamentation. Other cultivars may be less prone to gene mutation as naturally occurring plant phenol, ellagic acids and its derivatives, have been found to be antimutagenic (Pashin, et al., 1986; Smart et al., 1986).

Water-soluble polyphenols, tannins, are toxic to filamentous fungi, yeasts and bacteria (Scalbert, 1991) and inhibitory to viral reverse transcriptase (Nonaka et al., 1990). Their antimicrobial action is made possible by their capacity for protein complexation through hydrogen and covalent bonding and inactivation of microbial adhesions, enzymes and cell envelope transport proteins (Haslam, 1996; Stern et al., 1996). Condensed tannins bind to cell walls of ruminal bacteria preventing growth and protease activity (Jones et al., 1994) and inhibit insect growth on plants (Buttler, 1998). The consumption of tannins as

green teas (cf. Filipinos, Gertrudes, 2006) and wines prevents different illnesses (Serafini et al., 1994) and inhibits viral reverse transcriptase. The complete absence of tannin and phlobatannin in 'Sunray' followed by negative result for phenol was, thus, consistent for this cultivar.

Essential oils, terpenes, which give fragrance to plants, were present only in 'Sunray', thus, increasing its ornamental value above other cultivars. They exhibit antiameobic (Ghoshal et al., 1996), antibacterial (Amaral et al., 1998), antifungal (Rana et al., 1997) and anti-HIV (Fujioka et al., 1994) activities possibly through membrane disruption of the microorganisms by their lipophilic compounds. Terpenes may thus restore the loss of bioactivity in 'Sunray' due to lack of tannins and phenols.

Natural purgatives, anthraquinones, were absent in 'Spirale'. They are basically used as dyes, which could contribute to colour differences between this cultivar and others.

On the strength of the loss of essential bioactive chemical constituents from 'Spirale' (anthraquinones and cardenolides), 'Sunray' (tannin and phenol) and the presence of terpene producing enzymes in 'Sunray' only, both are considered to have reasonably diverged biochemically and physiologically from the rest of cultivars. They could, therefore, be taxonomically separated and raised to subspecific levels. *Sonchus* had been delimited from related genera, *Embergeria*, *Babcockia* and *Taekholmia* due to absence of luteolin- and apigenin 7-O rutinoside in addition to weak concentrations of coumarins and luteolin 7 glucoside (Mansour et al., 1983).

Phytopigmentation experiment in these cultivars is better carried out with the fresh leaf samples than dry because of the proximity of its values to the reference values. The relative constancy of Rf values for carotene and chlorophyll b indicated that they are better markers for pigmentation in this species compared to the other pigments. Food crops containing carotenoids, anthocyanins and other flavonoids were believed to function as 'chemopreventers' from cancer and reduction of cardiovascular disease (Stavric, 1994, Balch and Balch, 2000).

Chromosome counts ranging from $2n = 24 - 72, 48, 54 - 58, 60 - 64, 72, 80, 82, 96, 100, 106, 108, 112, 116, 120$ and 124 have been reported for different varieties of *Codiaeum* from India and the Philippines (Sharma and Bal, 1958; Pancho and Hilario, 1963; Datta, 1967; Gill et al., 1970; Chikkannaiah and Gayatri, 1978; Trivedi, Murkherjee and Trivedi, 1984; Chennaveeraiah and Wagley, 1985; Gayatri and Sunnita, 1991). The present reports of $2n = 30$ (Sunray), 24 (Royal-like) and 60 (Spirale, Royal, Broad Spotted Guinea and Punctatum) from Nigeria are additional. Cultivars 'Sunray' and 'Royal-like' have significantly reduced chromosome number. *C. variegatum* exhibit great diversity of morphological cha-

racteristics and is known to mutate spontaneously (Esan et al., 2005). Genetic mutation and chromosomes variability could account for the wide variation within the plant species. Somatic chromosome instability has been attributed to endoreduplication, irregular segregation, spindle multipolarity and unusual nucleus shape observed in dividing cells (Bruce, 1943; Boldrini et al., 2003).

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