

Review

African indigenous plants with chemotherapeutic potentials and biotechnological approach to the production of bioactive prophylactic agents

E. Olatunde Farombi

Drug Metabolism and Toxicology Unit, Department of Biochemistry, College of Medicine, University of Ibadan, Ibadan Nigeria. E-mail: olatunde_farombi@yahoo.com or ofarombi@skannet.com. Fax: 234-2-8103043 or 8103118.

Accepted 25 November 2003

Undoubtedly medicinal plants are relevant in both developing and developed nations of the world as sources of drugs or herbal extracts for various chemotherapeutic purposes. Also the use of plant-derived natural compounds as part of herbal preparations as alternative sources of medicaments continues to play major roles in the general wellness of people all over the world. The African continent contains some of the richest biodiversity in the world, and abounds in plants of economic importance and plants of medicinal importance which when developed would reduce our expenditure on imported drugs to meet our health needs. Herbal-based and plant-derived products can be exploited with sustainable comparative and competitive advantage. This review presents some indigenous African plants with chemotherapeutic properties and possible ways of developing them into potent pharmacological agents using biotechnological approaches.

Key words: Antioxidants, chemotherapy, medicinal plants, biotechnology, tissue culture, phytochemicals, prophylaxis, african plants, herbs, natural products.

INTRODUCTION

Higher plants, as sources of medicinal compounds continue to play a dominant role in maintenance of human health since antiquities. Over 50% of all modern clinical drugs are of natural product origin (Suffness and Douros, 1982) and natural products play an important role in drug development programs of the pharmaceutical industry (Baker et al., 1995; Cordell, 1995). In developing countries, especially in rural contexts people usually turn to traditional healers when in diseased conditions and plants of ethnobotanical origin are often presented for use.

Investigations into the chemical and biological activities of plants during the past two centuries have yielded compounds for the development of modern synthetic organic chemistry and the emergence of medicinal chemistry as a major route for the discovery of novel and more effective therapeutic agents (Roja and Rao, 2000).

Presently, many scientists and organizations are in search of traditional remedies as alternate medicine (Cortella and Pocheltino, 1999, OMS, UICN and WNF, 1993). Thus many plants that are used in traditional practice are sold in a rounded urban settlement to meet the need of a public desire for panaceas which has resulted in the industrialization and large scale production of a great number of products of botanical origin widely consumed.

The African continent is one of the continents endowed with the richest biodiversity in the world, with an avalanche of many food plants used as herbs, health foods and for therapeutic purposes. This is largely due to the geographical spread spanning a land mass of approximately 216, 634,000 hectares of closed forest areas. Over 5,000 different species of plant substances have been recognized to occur in these areas, and many

of them have been found to be useful in traditional medicine for prophylaxis and cure of diseases (Iwu, 1993). This great biodiversity therefore offers economic promise and in the rapidly emerging biotechnology industry.

In spite of the heterogeneous nature of the continent and a deluge of information on the composition and biological activity of many plant substances, there has been little effort devoted to the development of chemotherapeutic and prophylactic agents from these plants. In view of the emerging competitive world, therefore the evaluation of the constituents, pharmacological properties, detailed screening of bioactive substances for chemotherapeutic purposes are urgently warranted.

In this review, an overview of selected plant substances with potential chemotherapeutic values and some biotechnological approaches for the development and production of active substances with potent pharmacological effects is presented

***Garcinia kola* AND KOLAVIRON**

Garcinia Kola is a medium sized tree found in moist forest and widely distributed throughout west and central Africa. The nut is highly valued in these countries for its edible nuts (Hutchinson and Dalziel 1956). The seed commonly known as bitter kola is a masticatory and is a major kola substitute offered to guests at home and shared at social ceremonies. The seeds are used in folk medicine and in many herbal preparations for the treatment of ailments such as laryngitis, liver disorders, bronchitis (Iwu 1982). *G. kola* contains a complex mixture of biflavonoids, prenylated benzophenones and xanthenes (Terashima et al., 1995; Terashima et al., 1999; Hussain et al., 1982). Recently, two new chromanols, garcinoic acid, garcinal, together with δ -tocotrienol was reported (Terashima et al., 2002). The antioxidant activities were 1.5 times that of *dl*- α -tocopherol by the bleomycin-Fe assay (Terashima et al., 2002).

The biflavanones are the predominant compounds in *Garcinia kola* and GB-1, GB-2 and kola flavanones are the major components of kolaviron (Iwu, 1985). Kolaviron has been reported to significantly prevent hepatotoxicity induced by several hepatotoxic agents such as phalloidin, thioacetamide and paracetamol. (Iwu et al., 1987; Akintonwa and Essien, 1990). We have also reported its chemopreventive effects against carbon tetrachloride, 2-acetyl amino fluorine (2-AAF), Aflatoxin B1, and potassium bromate (Farombi et al., 2000a; Farombi, 2000a; Farombi et al., 2002a). These findings indicate that kolaviron may protect against carcinogen and drug-induced oxidative and membrane damage and as such may be relevant in the chemotherapy of liver and kidney diseases.

Biochemical mechanisms of action of kolaviron

Kolaviron had earlier been speculated to elicit its protective action on liver by acting as membrane stabilizer (Iwu et al., 1990). Braide (1991) reported the possible Interference of kolaviron with hepatic drug metabolism. However, these studies did not elucidate the mechanisms of action of kolaviron. We were therefore motivated to study the mechanism of antihepatotoxic effect of kolaviron.

Effect of kolaviron on xenobiotic metabolizing enzymes

We investigated using carbontetrachloride (CCl_4), a known liver toxic compound, the mechanisms by which kolaviron protects against tissue damage. The hepatotoxicity of CCl_4 is believed to require prior biotransformation in a cytochrome P450 catalysed reaction to trichloromethyl free radicals. These radicals either directly or before transformation to trichloromethyl peroxy radicals interact with cellular components such as lipids, proteins, nucleic acids and heme to induce damage (Slater 1984, Castro and Castro 1996).

Studies on the effect of kolaviron on rats treated with carbontetrachloride showed the preservation of the activities of phase 1 drug metabolizing enzymes such as aniline hydroxylase, aminopyrine *N*-demethylase, ethoxyresorufin *O*-deethylase and *p*-nitroanisole *O*-demethylase (Farombi, 2000a) while administration of kolaviron alone did not alter the activity of these enzymes. Our results further show that treatment of rats with kolaviron resulted in a marked elevation in the activity of uridyldiphosphoglucuronosyl transferase (UDPGT) and glutathione *S*-transferase (GST) indicating the ability of kolaviron to induce drug detoxifying enzymes (Farombi, 2000a). On the basis of our studies, it can be postulated that kolaviron may exert its protective action against oxidative damage to enzyme proteins and biomolecules by possibly scavenging reactive intermediates produced by the compounds via conjugation with glutathione or glucuronic acid with enhanced activity of GST and UDPGT respectively.

***In vitro* antioxidant action of kolaviron**

We investigated the antioxidant and scavenging activity of kolaviron in a range of established *in vitro* assays involving reactive oxygen species (Aruoma et al., 1990, 1993) Our data indicate a concentration dependent inhibition of hydrogen peroxide by kolaviron. Kolaviron (1.5mg/ml) elicited 85% inhibition of H_2O_2 and was more effective than BHA, β -carotene and compares with α -tocopherol (Farombi et al., 2002b). Also kolaviron at a concentration of 1 mg/ml significantly scavenged

superoxide generated by phenazine methosulfate NADH (PMS-NADH) (Farombi et al., 2002b). In our study, kolaviron inhibited the oxidation of deoxyribose. The IC_{50} for kolaviron and BHA were 0.3 and 3.3 mM respectively. The second order rate constant for the reaction of kolaviron and BHA with $OH\cdot$ were $1.1 \times 10^{10} M^{-1} S^{-1}$ and $2.4 \times 10^9 M^{-1} S^{-1}$ respectively.

Hydroxyl radical is highly reactive oxygen centered radical, which attacks all proteins, DNA, polyunsaturated fatty acids in membranes and almost any biological molecule it touches (Aruoma, 1999). The ability of kolaviron to scavenge hydroxyl radicals by inhibiting the oxidation of deoxyribose may relate directly to prevention of the propagation of the process of lipid peroxidation *in vivo* (Farombi et al., 2000a, Farombi, 2000a).

Furthermore our studies demonstrate that kolaviron inhibited the peroxidation of rat liver microsomes and this effects increase linearly with increasing concentration of kolaviron and the oxidizable substrate. Recent studies have demonstrated the ability of kolaviron to inhibit intracellular ROS induced by H_2O_2 in HepG 2 cells detected as 2, '7' -dichlorodihydrofluorescein diacetate (DCF) fluorescence (Nwankwo et al., 2000). The human cell line employed in this study was HepG 2, derived from a well-differentiated human hepatoblastoma, which retains many of the morphological characteristics of liver parenchyma cells (Eddy et al., 1987). Thus, the ability of kolaviron to act as antioxidant in this cell indicates its potential role in the chemoprevention of chemically-induced genotoxicity

Molecular mechanism of action of kolaviron

Nwankwo et al. (2000) reported the ability of kolaviron to inhibit Aflatoxin B₁ induced genotoxicity in a human liver-derived cell line (HepG 2). This cell line has been routinely applied to studying metabolic activation of carcinogens to genotoxic metabolites (King et al., 1999). This model therefore allows reasonable inferences as to the situation *in vivo*. In this study AFB₁, a potent hepatocarcinogen in experimental animals (Wogan et al., 1974) and human carcinogen (IARC, 1993) was used as a model genotoxic compound.

kolaviron specifically induced CYP3A4 gene transcript by 3.7 fold at a concentration of 90 μ mol/l using northern blotting analysis (Nwankwo et al., 2000). Similarly GST isozyme α -1 and α -2.2 were also induced by 2.2- and 2.5 fold levels respectively for their messages as determined by Reverse transcription polymerase chain reaction (RT-PCR) and northern analysis and 2 fold increase in GST α protein by western blotting. These findings suggest that kolaviron may be relevant in inhibiting AFB₁-induced genotoxicity and explains its possible molecular mechanisms of action. Further studies indicate the ability of kolaviron to mitigate oxidative damage to DNA (Farombi et al., unpublished data).

RED PALM OIL

Red palm oil is produced from the fruit of the *Elaeis guineensis* tree and has been used for food for some 5000 years (MacFarlane et al., 1984). It is unique among edible oils and natural plant foods as it is the richest known natural source of β -carotene (7500 mol/l) (Manorama and Rukmini, 1991). The tree is widely cultivated particularly in Nigeria and other parts of Africa. It is cheaper than other edible oils and this makes it a promising source of vitamin A in a deficient population.

Palm Oil Carotenoids

Carotenoids are responsible for the characteristic colour of red palm oil. They are present at concentrations of 500-700mg/l in the crude oil (Cottrell, 1991). α and β -carotenes are the major components with γ -carotene, lycopene, lutein and zeaxanthin present in smaller amounts. Palm oil contains 80-90% of its carotenoids as β -carotene and α -carotene in 2:1 ratio respectively (Tan and Chu, 1991). These lipid soluble pigments have been isolated and identified in palm oil (Ng and Tan, 1988; Farombi and Britton, 1999a).

Antioxidant activities of palm oil carotenes

We examined the antioxidant activity of carotenoids extracted from palm oil in both organic solution as well as multilamellar liposomes. Oxidation of four carotenoids (α -carotene, β -carotene, lutein, and zeaxanthin) with 2, 2'-azobis (2,4-dimethyl valeronitrile) (AMVN)-generated peroxy radicals in organic solution, demonstrated that all the carotenoids showed a linear rate of decrease in concentration. β -carotene and α -carotene were the most rapidly oxidized whilst zeaxanthin and lutein exhibited the lowest rates of oxidation (Farombi and Britton, 1999a, Farombi, 2000b). The low reactivity of lutein and zeaxanthin may possibly be due to the presence of polar hydroxyl groups attached to them and not react in a non-polar organic solution. Chopra et al. (1993) similarly observed that the reactivity of lutein with free radicals in organic solution decreased with decreasing polarity of the solvent.

Studies have shown that in the presence of oxidizing agent, all carotenoids react rapidly, though the reactivity depends on some factors such as the length of the polyene chromophore and also on the nature of the functional groups (Britton, 1995). Recent studies however have demonstrated that the presence of functional groups affects the reactions of carotenoids with radicals more than the chromophore or the polyene chain. For instance in the reaction of β -carotene, zeaxanthin and isozeaxanthin with 2,2' azo-bis-isobutyronitrile (AIBN), β -carotene and zeaxanthin had similar rate of reaction but isozeaxanthin reacted slower

than either of the two. The three carotenoids have the same chromophore that is the nine conjugated double bonds of the polyene chain plus a contribution from the two double bonds of the β -rings (Woodall et al., 1997a). Clearly, the influence of the presence and position of the hydroxyl group at the 4-position modified the reactivity of the carotenoid suggesting that other factors than free-radical addition to the polyene chain or electron capture by the chromophore is involved (Woodall et al., 1997a).

We examined for the first time the antioxidant efficacy of α -carotene in egg yolk phosphatidyl choline solution. Our results indicate that AMVN induced peroxidation of phosphatidyl choline resulted in the formation of phosphatidyl choline hydroperoxide (PCOOH) and thiobarbituric acid reactive substances (TBARS) (Farombi and Britton, 1999a). The superiority of α -carotene to β -carotene as antioxidant may be due to the cis configuration on the β -ionone ring of α -carotene. This arrangement may presumably enhance the ability of α -carotene to better trap radical species than β -carotene. Similar effect had been observed in Fe^{3+} -ADP/NADPH and in paraquat/NADPH systems (Kim, 1990).

OTHER MEDICINAL PLANTS

Alchornea species

Alchornea laxiflora (Benth). Pax and Hoffman (Euphorbiaceae) is a forest understory tree of about 6m high growing in Nigeria. It is also found in the Cameroons and it is widespread in the central and southern tropical Africa. The leaves play important role in the preservation of kolanuts widely eaten in Nigeria. The stem and branchlets are also used in Nigeria as chewing sticks. Decoction of the leaves are used in the treatment and management of inflammatory and infectious diseases as well as an important component of herbal antimalarial formulations (Adewole, 1993).

Alchornia cordifolia a closely related species are used in the preparation of remedies for urinary, respiratory and gas to intestinal disorders (Iwu, 1993). Its antibacterial activity on *Staphylococcus aureus* and *Escherichia coli* was attributed to isopentenyl guanidine (Lamikanra et al, 1990). Phytochemical screening of the powdered leaf samples of *A. laxiflora* revealed the presence of alkaloids, cardiac glycosides saponins and phenolic compounds (Ogundipe et al., 1999). The presence of terpenoid compounds was recently discovered in the root samples of *A. laxiflora* (Farombi et al., 2003).

Pilot studies of the crude and purified fractions of the leaves and roots revealed the presence of potent anti-inflammatory and antimicrobial activities in the methanol extract (Ogundipe et al 1999). Subsequently, quercetin-7,4'-disulphate, quercetin, quercetin-3',4'-disulphate, quercetin-3,4'-diacetate, rutin and quercetrin were characterized from the methanolic leaf extract of *A. laxiflora* (Ogundipe et al 2001). The isolated compounds

were found to possess anti-microbial activity detected in Gram-positive, Gram-negative and fungal organisms (Ogundipe et al., 2001).

Vernonia amygdalina

Vernonia amygdalina (compositae) is a small shrub that grows predominantly in the tropical Africa. In Nigeria, the plant is locally called bitter leaf due to its bitter taste. The macerated leaves of the plant are used in making soup while the water extract serves as a tonic drink for the prevention of certain illnesses. The leaves have found relevance in traditional folk medicine as antihelmint, a laxative herb and an antimalarial as they are known as quinine substitute. *V. amygdalina* has been reported for its use by wild chimpanzees for the treatment of parasite-related diseases in Tanzania (Huffman and Seifu, 1989).

Several stigmasterane-type saponins such as vernoniosides A1, B1, A2, A3, B2, D3, A4 and C have been identified in the leaves (Ohigashi et al., 1991; Jisaka et al., 1992; Kamperdick et al., 1992). Phillipson et al. (1993) reported the antiplasmodial effects of some sesquiterpene and steroidal constituents of *V. amygdalina* and some were also effective against *Plasmodium falciparum* *in vitro*. The antioxidant activities of luteolin, luteolin 7-O, β -glucuronoside and luteolin 7-O- β -glucoside flavonoid compounds isolated from the leaves of *V. amygdalina* have been reported using coupled oxidation of β -carotene linoleic acid (Igile et al., 1994).

Earlier investigations on *V. amygdalina* showed that purified chloroform fractions identified as vernodaline, vernolide and vernomygdine elicited cytotoxic effects in human carcinoma nasopharynx cells with IC_{50} values of 1.8, 2.0 and 1.5 $\mu\text{g/ml}$ respectively (Kupchan et al., 1969) It was concluded that the activities were dependent on their possessions of the (α -methyl- γ -lactone group) as part of their structures. Subsequently Jisaka et al. (1993) demonstrated that vernodaline and vernolide elicited antitumoral activities in leukemia cells P-388 and C-1210 with IC_{50} values of 0.11 and 0.17 $\mu\text{g/ml}$ for vernodaline and 0.13 and 0.11 $\mu\text{g/ml}$ for vernolidee respectively. Recently Izevbigie (2003) isolated some peptides (edotides) from the aqueous extract of *V. amygdalina*. The peptides were shown to be potent inhibitor of mitogen-activated proteins kinases (MAPKs) which are crucial for breast tumor growth and also represents a key regulatory point for tumor growth. The antiestrogen breast cancer drug (tamoxifen) has also been shown to modulate MAPK activity (Atanaskova et al., 2002; Mandlekar and Kong, 2001). This indicates that edotides from *V. amygdalina* may be considered as alternative to tamoxifen. Furthermore, extracts from *V. amygdalina* have also been suggested to have cell growth inhibitory effects in prostate cancer cell line (PC-3) and no effect on normal human peripheral blood mononuclear cells (PBMC) (Izevbigie, 2003).

Mallotus oppositifolius

Mallotus oppositifolius (Euphorbiaceae) is a shrub of up to 13.5 cm long and 2.5–10 cm wide which grows in old forms of secondary forest and thickets. It also thrives in the savannah vegetation. It is used as herb for the treatment of dysentery and as a vermifuge. The leaves are ingredients of common antimalarial and anti-inflammatory remedies (Burkhill, 1994).

Phytochemical screening of *M. oppositifolius* revealed the presence of secondary metabolites such as alkaloids, phenols, flavonoids, anthraquinones and cardenolides. A higher concentration of these resides in the leaves than in the root (Farombi et al., 2001). Five hydrolysable tannins and cytotoxic phloroglucinol have been reported from the bark of *M. japonicus*, another mallotus species (Iwu, 1993).

Preliminary investigation in our laboratory indicates potent antioxidant and anti-inflammatory activities of extracts from the leaves of *M. oppositifolius*. Further studies are on to characterize the plant extract as antioxidant prophylactic agent.

Yam plant

Yam plants are classified under the genus *Dioscorea*, family Dioscoreaceae. The most important species of *Dioscorea* include *D. rotundata*, *D. alata*, *D. cayenensis*, *D. dumatrium*, *D. esculenta*, and *D. bulbifera*. Yams produce edible tubers bulbils or rhizomes which are of considerable economic importance (Ammirato, 1984). They serve as a major carbohydrate staple particularly in the western and Eastern part of Nigeria. Many wild species are of medicinal and pharmacological importance (Coursey, 1967). Analysis of the chemical components in the edible yam has revealed the occurrence of polyphenolic substances such as catechins, epicatechins, chlorogenic acids, leucoanthocyanidins and anthocyanins (Imbert and Seaforth, 1968, Martin and Ruberte, 1975). Studies on chemical reactions of yam indicate that the polyphenolic compounds in yam are capable of undergoing biotransformation by polyphenol oxidase present in the yam (Ozo, 1985) to brown polymeric compounds (Walker, 1995, Ozo, 1985). At the same time amino acids and proteins in the yam when heated can react non-enzymatically with sugars to form brown-coloured compounds commonly referred to as Maillard reaction products (Maillard, 1912). Data from our laboratory have indicated the protective effect of maillard reaction products against oxidative toxicity induced by some environmental carcinogens (Farombi et al., 1997, 1998; Farombi, 1998; Farombi et al 2000b). Further investigation revealed the presence of complex and maillard reaction products such as pyrazines and acetyl furans in the ethyl acetate fraction of the browned yam flour diet (Farombi et al., 2000c). These compounds

elicited significant and potent antioxidant activities in various models.

Hibiscus sabdariffa

Hibiscus Sabdariffa L belongs to the family malvaceae. The dried flowers of *H. sabdariffa* are a popular plant in Nigeria. They are used in cold beverages called 'Sobo'. Besides, the calyces of Hibiscus have been used in traditional medicine, also in Thailand and Mexico and they are rich in phenolic compounds with marked physiological activities (Resendiz-Lopez et al., 1998). Hibiscus flowers contain gossypetin, glucoside, bibiscin, hibiscus anthocyanin and Hibiscus protocatechuic acid, which may have diuretic and choleric effects, decreasing the viscosity of the blood, reducing blood pressure, and stimulating intestinal peristalsis (Ali and Salih, 1991).

Resendiz-Lopez et al. (1998) reported that the dried flowers of *H. sabdariffa* is a functional food with a chemopreventive capacity. Hibiscus protocatechuic acid (PCA), a simple phenolic compound isolated from *Hibiscus sabdariffa* L. was shown to significantly decrease the leakage of lactate dehydrogenase (LDH) and alanine transaminase (ALT) and the formation of malondialdehyde (MDA) induced by tert-butylhydroperoxide (t-BHP) in rat primary hepatocytes (Tseng et al., 1996).

Investigation also revealed that the crude extracts of the dried flowers of *H. sabdariffa* L. has strong antioxidant potential as they inhibited xanthine oxidase activity, formation of MDA and scavenged 1, 1-diphenyl 1-2-picrylhydrazide (DPPH) free radicals most effectively. The extract inhibited the unscheduled DNA repair synthesis induced by t-BHP in the rat hepatocyte cultures (Tseng et al., 1997). They revealed that the dried flowers extract of *H. sabdariffa* protect rat hepatocytes from t-BHP-induced cytotoxicity and genotoxicity by different mechanisms.

Hibiscus anthocyanins (HAs), isolated from the dried flowers of *H. sabdariffa* L. were studied for antioxidant bioactivity (Wang, et al; 2000). They reported the ability of HAs to quench the free radicals of DPPH, decrease the leakage of LDH and the formation of MDA, lower the serum levels of hepatic enzyme and reduced oxidative damage.

The pigments contained in the flowers of Hibiscus species are anthocyanins such as cyanidin-3-glucoside and delphinidine-3-glucoside (Du and Francis, 1973; Nakamura et al., 1990) and have been used in food manufacture. It has been reported that anthocyanins showed antioxidant activity in a liposomal system (Tsuda et al., 1996): however, its bioactivity is uncertain in intact cell and *in vitro* system.

It has also been demonstrated that the PCA, isolated from *H. sabdariffa*, present in fruits, vegetables and nuts is an efficacious agent in reducing the carcinogenic

action of diethylnitrosamine in the liver (Tanaka et al., 1993), 4-nitroquinone 1-oxide in the oral cavity (Tanaka et al., 1994), N-methyl-N-nitrosourea in glandular stomach tissue (Tanaka et al., 1995). But the mechanism by which PCA exerts its suppressing effect on chemical carcinogenesis is unknown.

BIOTECHNOLOGICAL APPROACH TO THE PRODUCTION OF PLANT FOODS

Keeping in pace with modern demand, it has become highly imperative to regenerate, multiply and conserve plants with desirable traits. Although most plants have natural means of doing this, but they have not been able to meet the demands of man. Application of biotechnological tools to the regeneration and sustenance of plant food substances have remained the exclusive preserve of the western world, though a few developing countries in central and South America and Asia have tried to adopt the techniques. In Africa, the application of these techniques is still in the infancy stage thus, there is the urgent need to apply these modern biotechnological tools to all economic tree crops for the multiplication of superior plant individuals. Some suggested biotechnological tools for the production of yam and oil palm is hereby presented.

Yam

The near absence of modern biotechnological approaches to yam propagation has resulted in a low presence for yam in international trade compared to other crops such as potatoes. Earlier investigations by Correl et al. (1955) on wild yams with medicinal and pharmacological properties and by Njoku (1963) on edible yams succeeded in the regeneration of yams from leafy vine cuttings. Successful propagation of wild yam and edible yam through vine nodal explants has also been reported (Vasanthakumar, 1981; Okonkwo et al., 1986). *In vitro* culture of defoliated nodal explants on suitable agar nutrient media has been used also in the regeneration of edible and medicinal yams. Tissue culture procedures have been adopted for clonal propagation of an elite yam cultivar. The culture of excised meristem has been used for large-scale clonal propagation (Mantell et al., 1980). Plants which are produced clonally by nodal or meristem culture show a high degree of uniformity (Ammirato, 1984). The use of callus and cell cultures from various plant organs for the expression of totipotency via somatic embryogenesis may prove useful for yam propagation. The use of 2,4 dichlorophenoxy acetic acid as the auxin in the culture medium has been shown to initiate proliferation and maintenance of unorganized growth during subculture (Okezie and Okonkwo, 1992). Transfer of the material to a secondary medium with 2,4-D, with lower

concentration of 2, 4-D coincided with the appearance of embryo or plants (Ammirato, 1984; Okezie and Okonkwo, 1992). Very recently the National root crops research institute (NRCRI) in Nigeria has developed novel tissue culture protocols for yam and other tree crops (Kwong-Ndung and Misari, 2002).

Palm oil

The application of tissue culture to oil palm production has been on for three decades since the first successful regeneration of plantlets from leaf tissue was reported by Rabechault et al. (1972). Ever since, several investigations have improved upon this method. The technique of producing somatic cell embryos through a callus intermediary have been used (Durand-Gasselien et al., 1990).

The use of explants for the regeneration of oil palm using tissue culture procedure has been described and summarized by Esan (1992). Explants were obtained from shoot apices of unopened young leaves of trees aged 3-25 years and cultured on Murashige-skoog (Ms) medium augmented with an auxin usually 2, 4-D. After 60 days primary callogenesis occurred, usually from secondary veins. The callus was allowed to multiply for about 6 months to produce secondary callus. The calluses produced somatic embryos.

Other candidates

In vitro multiplication and vegetative propagation via tissue culture and somatic embryo genesis methods have been successfully applied to the production of some medicinal herbs in different places. *Tinospora cordifolia* Miers (Menispermaceae), a medicinal plant of Indian subcontinent, has been used for treating jaundice, rheumatism and urinary disorders (Reddy et al., 2003). The plant also possesses anti-inflammatory, anti-allergic and immunomodulatory properties. *In vitro* multiplication of *T. cordifolia* via direct somatic embryogenesis using leaf explants of 15-days-old plants on MS medium supplemented with 2, 4-D (0.5 mg/l) and glutamine (20 mg/l) produced viable somatic embryo (Reddy et al., 2003). The same method has been applied to the production *Mallotus repandus* a potential medicinal plant for anti-inflammatory drug development (Kaewsuwan et al., 2003), *Zanthoxylum piperitum* DC, an important chromatic and medicinal plant (Hwang et al., 2003), *Hemidesmus indicus* R. Br, highly valued in the traditional systems of medicine for abdominal tumour fractures and skin diseases (Misra et al 2003) and *Typhonium trilobatum* an antifilarial medicinal plant (Das, 2003). Embryo culture for germinating *H. sabdariffa* is being developed at the International institute of tropical agriculture (IITA) in Nigeria (Sarumi, 2002). Callus formation with root growth has been obtained from

Eugenia sp. and shoot formation from the nodal culture of an exotic fruit (passion fruit) has also been obtained (Sarumi, 2002).

Production of prophylactic agents

Chemotherapeutic agents continue to play pivotal roles in the general wellness of individuals and management of human clinical conditions. However the problem of multi drug resistance and overt toxicities of many drugs currently in use have underpinned the search for non-toxic, effective, easily assessable, natural compounds from plant sources. The discovery of natural anticancer agents with promising biological activity has demonstrated the significance of medicinal plants in nature (Roja and Rao, 2000). The limited availability of these compounds indicate the need to collect plants from the wild. Furthermore, limited plant materials has posed difficulties in securing enough of the compounds for pharmacological trials and chemotherapeutic purposes. Total chemical synthesis is too complicated to produce sufficient amounts of these natural compounds and as a result, tissue culture has been suggested as an alternative method for the production of these phytochemicals.

Application of tissue culture in medicinal plants has proved to be useful in the production of therapeutic compounds. With this vital tool, the desired constituents may be obtained during a short time period without the destruction of the entire plant.

Some cancer chemotherapeutic agents isolated from plant sources have been produced in large quantities using tissue culture. For example, Paclitaxel, a distinctive diterpenoid has become one of the most important lead compounds to emerge from the screening of natural products in recent years (Kingston, 1992). Paclitaxel has demonstrated significant activity against various leukemias, the Walker 256 carcinosarcoma, Sarcoma 180, and the Lewis lung tumor (Wan et al., 1971). The chemical synthesis of paclitaxel molecule is difficult to achieve due to the complex structure of the molecule, making a commercial source of Paclitaxel unlikely (Roja and Rao, 2000). Hence plant tissue culture has been suggested as an alternative method to provide large amounts of Paclitaxel and related compounds (Roja and Rao, 2000) and the production of Paclitaxel from cell cultures has been patented (Christen et al., 1991).

Ochrosia elliptica contain the alkaloids ellipticine 9-methoxyellipticine, isoreserpiline and elliptinine (Loder, 1966). Ellipticine, 9-methoxyellipticine, have displayed tumor inhibitory activity due to the intercalation of the alkaloid with the DNA (Rao and Roja, 2000). In tissue culture studies on *O. elliptica*, callus cultures were easily developed from leaf explants on B5 medium supplemented with kinetin (0.1 mg/liter), 2, 4-dichloro phenoxyacetic acid (10 mg/liter) and casein hydrolysate

(0.1%). The granular calli of *Ochrosia elliptica* obtained in this medium accumulated ellipticine and reserpiline during the stationary growth phase. The yield of ellipticine in the tissue during 10 to 15 different passages was comparable with the levels in leaves of the intact plant (Rao and Roja, 2000).

Plants with potent antioxidant activity which play modulatory role in chemical carcinogenesis can also represent an important alternative source for natural antioxidants by biotechnological approach. Bahorun et al. (2002) demonstrated the production of polyphenolic antioxidants from 10 year oil *Grataegus monogyna* calli within a subculture period. *Grataegus monogyna* has long been used as a folk medicine and is widely utilized in pharmaceuticals mainly because of its neuro- and cardiosedative actions and its low toxicity. It's pharmacological efforts are mainly attributed to poly phenolics.

CONCLUSION AND FUTURE PERSPECTIVES

Agrobiotechnology undoubtedly is in place in Africa but effort should be made to develop and sustain modern biotechnology by harnessing the rich biodiversity in Africa. For instance the IITA has developed methods for tissue culture and micropropagation of *Musa* species. Nigerian Institute for Oil palm Research (NIFOR) Benin has developed protocol for tissue culture and micro propagation of oil palm (Kwon-Ndung and Misari, 2002). However, little or no effort has been directed towards the use of biotechnological techniques for the production of chemotherapeutic agents, which can be used to fight some African endemic diseases such as malaria, liver cancer and other tropical related diseases. In Japan, Thailand, India and countries in the developed world such initiatives are in place. Presently many countries in Africa have policies directed at developing biotechnology capabilities, through funding of projects, training of researchers and creation of specialized research institutes (Sonaiya et al., 2002).

These policies represent the beginning of development of biotechnology capabilities. Active research and development is imperative for understanding the basic science involved in monitoring international scientific trends and events and in training researchers. Collaborations by different research centers in the continent with international bodies involved in the promotion of biotechnology activities should be encouraged. Because of the general availability of medicinal plants and the usually low production costs, low toxicity and limited side-effects, WHO in addition to advocating the use of traditional remedies has sponsored multicentered projects in the search for new drugs from plants, especially in plants indigenous to the tropical African in the last few years.

Plant substances continue to serve as exclusive

source of drugs for the majority of the world population and several plant based drugs are in extensive clinical use (Rao and Roja, 2000). Africa contains some of the richest sources of biodiversity in the world. Herbal-based and plant-derived products can be exploited with a sustainable comparative and competitive advantage. Very recently, science and technology are supporting bio-prospecting by taking the pain to analyze how indigenous people use plants for health care. The importance of plant drugs in chemotherapy of various diseases justifies the additional laboratory and classical research into the search and study of potentially pharmaceutically useful plant materials.

In spite of the biodiversity in tropical Africa, many rich and useful plants suffer severe limitations and setbacks and have not garnered international prominence. This is due to the dearth of state-of-art research facilities necessary to extract, isolate and elucidate the chemical components in the plants. Therefore modern spectroscopic facilities should be procured through concerted efforts involving, research institutes, universities, industries, national governments and international organizations. These equipments will play crucial role in modern characterization of plant-derived substances as chemotherapeutic agents.

The limited availability of some plant materials and the need to protect endangered species, however, may impose restriction on the use of some plant species. Thus the future availability of the plant metabolites may well depend upon large scale biotechnological approaches for the production of bioactive prophylactic agents.

ACKNOWLEDGMENTS

The author is grateful to the University of Ibadan (Senate Research Grants), Nigeria and the World Bank Research Fund for support. Mr. Mike Segilola is thanked for excellent secretariat assistance.

REFERENCES

- Adewole AA (1993). Personal communication with local traditional medical practitioner in Ibadan, Nigeria.
- Akintonwa A, Essien AR (1990). Protective effects of *Garcinia kola* seed extract against paracetamol-induced hepatotoxicity in rats. *J. Ethnopharmacol.* 29: 207-211.
- Alli MB, Salih WM (1991). Investigation of the antispasmodic potential of *Hibiscus sabdariffa* calyces. *J. Ethnopharmacol.* 31: 249-257.
- Ammirato PV (1984). Yams. In Handbook of plant and cell culture pp 327-352; Vol 3: crop species ed. PV Ammirato. DA Evans and Y Yamada. Macmillan, New York, USA.
- Aruoma OI (1999). Free radicals and International nutrition. *Asia Pacific J. Clin. Nutr.* 8: 53-63.
- Aruoma OI, Evans PJ, Kaur H, Sutcliffe L, Halliwell B (1990). An evaluation of the antioxidant and potential pro-oxidant properties of food additives and Trolox C, vitamin E, and probucol. *Free Rad. Res. Commun.* 10: 143-157.
- Aruoma OI, Murcia A, Butler J, Halliwell B (1993). Evaluation of the antioxidant actions of gallic acid and its derivatives. *J. Agric. Food Chem.* 41 1880-1885.
- Atanaskova N, Keshamouni VG, Krieger JS, Schwartz JA, Miller F, Reddy KB (2002). MAP kinases/estrogen receptor cross-talk enhances estrogen-mediated signaling and tumor growth but does not confer tamoxifen resistance. *Oncogene* 21: 4000-4008.
- Bahorun T, Aumjaud H, Ramphul H, Rycha M, Luximon-Ramma A, Troth F, Aruoma OI (2002). *Crataegus monogyna* (Hawthorn) callus extracts: A rich source of antioxidant phenolics. Abstracts 3rd International conference of the Society of Free Radical Research-Africa. CapeTown, South Africa 8-11 October 2002, p. 9.
- Baker JT, Borris RP, Carte B, Cordell GA, Soejarto DD, Cragg GM, Gupta MP, Iwu MM, Madulid DR, Tyler VE (1995). Natural product drug discovery and development: New perspective on international collaboration. *J. Nat. Prod.* 58: 1325-1357.
- Braide, V.B. (1991). Inhibition of drug metabolism by flavonoid extract (Kolaviron) of *Garcinia kola* seeds in the rat. *Phytotherapy Res.* 5 38-40.
- Britton, G (1995). Structure and properties of carotenoids in relation to function. *Fed. Am. Soc. Exp. Biol. J.* 9: 1551-1558.
- Burkhill HM (1994). The useful plants of West tropical Africa, Royal botanic gardens, Kew Vol 2: 144-150.
- Castro GD, Castro JA (1996). Tryptophan reaction with free radicals arisen from carbon tetrachloride in a model system. A mass spectrometric study. *Redox Report* 2: 47- 54.
- Chopra M, Wilson RL, Thurnham DL (1993). Free radical scavenging of lutein in vitro. *Annals New York Acad. Sci.* 1: 246-249.
- Christen AA, Gibson DM, Blandt J (1991). Production of taxol or taxol like compounds in cell culture. United States patent 5: 019, 504.
- Cordell GA (1995). Changing strategies in natural products chemistry. *Phytochemistry* 40: 1585-1612.
- Correl DS, Schubert BG, Gentry HS, Kawley WO (1955). The search for plant precursors of cortisone. *Econ. Bot.* 9: 307-375.
- Cortella AR, Pocheltino ML (1999). Plants employed for infusions in urban areas of Argentina. *Pharm. Biol.* 37: 97-104.
- Cottrell RC (1991). Introduction: nutritional aspects of palm oil. *Am. J. Clin. Nutr.* 53: 989S-1009S.
- Coursey DG (1967). Yams Longman, London pp 230-245
- Das P (2003). In vitro regeneration of *Typhonium trilobatum* Schott: A potential Antifilarial plant via. Somatic embryogenesis. embryogenesis Abstract, 3rd World Congress on Medicinal and aromatic plants for human welfare Chiang Mai, Thailand, 280 p.
- Du CT, Francis FJ (1973). Anthocyanins of Roselle (*Hibiscus sabdariffa* L.). *J. Food Sci.* 38: 310-312.
- Durand-Gassselin T, LeGeun V, Konan K, Duval Y (1990). Oil palm (*Elaeis guineensis* Jacq) plantation in Cot d'Ivoire, obtained in vitro culture. *Oleagineux* 45: 1-11.
- Eddy EP, Howard PC, McCoy GD, Rosenkranz HS (1987). Mutagenicity, unscheduled DNA synthesis, and metabolism of 1-nitropyrene in the human hepatoma cell line HepG2. *Cancer Res.* 47: 3163-3168.
- Esan EB (1992). Status of in vitro regeneration of tropical plantation crops .In: Biotechnology: Enhancing research on Tropical Crops in Africa. Ed Thottappilly G, Monmti LM, Mohan Raj DR, Moore AW . CTA.IITA co-publication. IITA, Ibadan, Nigeria pp 157-164.
- Farombi EO (2000a). Mechanisms for the hepatoprotective action of kolaviron: Studies on hepatic enzymes, microsomal lipids and lipid peroxidation in carbon tetrachloride-treated rats. *Pharmacol. Res.* 42: 75-80.
- Farombi EO, Britton G (1999a). Antioxidant activity of palm oil carotenes in organic solution: effects of structure and chemical reactivity. *Food Chem.* 64 : 315-321.
- Farombi EO, Akuru TO, Alabi MC (2002a). Kolaviron modulates cellular redox status and impairment of membrane protein activities by potassium bromate (KBrO₃) in rats. *Pharmacol. Res.* 45: 63-68.
- Farombi EO, Akanni OO, Emerole GO (2002b). Antioxidant and scavenging activities of flavonoid extract (kolaviron) of *Garcinia kola* seeds in vitro. *Pharm. Biol.* 91: 129-134.
- Farombi EO, Britton G, Emerole GO (2000c). Antioxidant and partial characterisation of extracts from browned yam flour. *Food Res. Int.* 33: 493-499.
- Farombi EO, Nwankwo JO, Emerole GO (1997). Possible modulatory effect of browned yam flour diet on chemically induced toxicity in the

- rat. Food Chem. Toxicol. 35: 975-979.
- Farombi EO, Nwankwo JO, Emerole GO (1998). Effect of methanolic extract of browned yam flour diet on 7,12 Dimethylbenzanthracene (DMBA) and 3-methylcholanthrene (3-MC)-induced toxicity in the rat. Proceedings of the Federation of African Society on Biochemistry and Molecular Biology.1: 5-10
- Farombi EO, Nwankwo JO, Emerole GO (2000b). Modulation of carbontetrachloride-induced lipid peroxidation and xenobiotic-metabolizing enzymes in rats fed browned yam flour diet. Afr J. Med. Sci. 29: 64-68.
- Farombi EO, Ogundipe OO, Moody JO (2001). Antioxidant and anti-inflammatory activities of *Mallotus oppositifolius* in model systems. Afr. J. Med. Sci. 30: 213-215.
- Farombi EO, Tahnteng JG, Agboola O, Nwankwo JO, Emerole G.O (2000a). Chemoprevention of 2-acetylaminofluorene-induced hepatotoxicity and lipid peroxidation in rats by kolaviron- A *Garcinia kola* seed extract. Food Chem. Toxicol. 38: 535-541
- Farombi EO (2000 b). Antioxidant potency of alpha (α)-carotene: Relevance and potential use in the food industry. Food Safety Objectives Conference, December 4-5, Georgetown University, Washington DC, USA.
- Farombi EO, Britton G (1999b). Antioxidant activity of palm oil carotenes in peroxy radical-mediated peroxidation of phosphatidyl choline liposomes. Redox Report 4 61-68
- Farombi EO, (1998). Modulation of hepatic xenobiotic-metabolizing enzymes by browned yam flour diet in rats fed bromobenzene. Proc. Fed Afr. Soc. Biochem. Mol. Biol. 1: 11-14.
- Farombi EO, Ogundipe OO, Uhunwagho ES, Adeyanju MA, Moody JO (2003). Antioxidant properties of extracts from *Alchornea laxiflora* (benth) Pax and Hoffman. Phytotherapy Res. 7: 713-716.
- Huffman MA, Seifu M (1989). Observations of the illness and composition of a medicinal plant *Vernonia Amygdalina* by wild Chimpanzee in the Mahale mountains, Tanzania. Primates 30: 51-63.
- Hussain RA, Owegby AG, Parimoo P, waterman PG (1982). Kolanone, a novel polyisoprenylated benzophenone with antimicrobial properties from the fruit of *Garcinia kola*. Planta Med. 44: 78-81.
- Hutchinson J, Dalziel JM (1956). Flora of West Tropical Africa, 2nd edn. Vol 1. P 295. HMSO, London.
- Hwang SJ, Na MS, Pyo BS, Hwang B (2003). An efficient in vitro propagation of *Zanthoxylum piperitum*. Abstract, 3rd World Congress on Medicinal and aromatic plants for human welfare Chiang Mai, Thailand, p. 284.
- IARC (1993). Evaluation of carcinogenic risks to human. Some naturally occurring substances: Food items and constituents, Heterocyclic aromatic amines and mycotoxins IARC Monographs 56 362
- Igile GO, Oleszek W, Jurzysta M, Burda S, Fafunso M, Fasanmade AA (1994). Flavonoids from *Vernonia amygdalina* and their antioxidant activities. J. Agric. Food Chem. 42: 2445-2448.
- Imbert M P, Seaforth C (1968). Anthocyanins in *Dioscorea alata*. Experientia 24: 445-447.
- Iwu MM (1982). Traditional Igbo Medicine. Pp 104. Institute of African Studies, University of Nigeria, Nsukka.
- Iwu MM (1985). Antihepatotoxic constituents of *Garcinia kola* seeds. Experientia 41: 699-670.
- Iwu MM (1993). Hand book of African medicinal plants Pp 1 CRC press, Inc Florida.
- Iwu MM, Igboko OA, Okunji CO, Tempesta S (1990). Antidiabetic and aldose reductase activities of biflavonones of *Garcinia kola*. J. Pharm. Pharmacol. 42: 290-292.
- Iwu MM, Igboko OA, Onwuchekwa U, Okunji CO (1987). Evaluation of the antihepatotoxicity of the biflavonoids of *Garcinia kola* seeds. J. Ethnopharmacol. 21: 127-142.
- Izevbigbe EB (2003). Discovery of water soluble anticancer agents (Edotides) from a vegetable found in Benin city, Nigeria. Exp. Biol. Med. 228: 293-298
- Jisaka M, Ohigashi H, Takagaki T, Nozaki H, Tada T, Hirota M, Irie R, Huffman MA, Nishida T, Kaji M, Koshimizu K. (1992). Bitter steroid glucosides, Vernosiosides A1, A2 and A3 and related B1 from a possible medicinal plant *Vernonia amygdalina*, used by wild chimpanzee. Tetrahedron 48: 625-630.
- Jisaka M, Ohigashi H, Takagawa K, Nozaki H, Hirota M, Irie R, Huffman MA, Koshimizu K. (1993). Steroid glucosides from *Vernonia amygdalina*. A possible chimpanzee medicinal plant. Phytochemistry 34: 409- 413.
- Kaewsuwan S, Soonthorncharenon N, Prathanturug S (2003). In vitro propagation of *mallotus repandus* (Wild.) Muell. Arg. embryogenesis Abstract, 3rd World Congress on Medicinal and aromatic plants for human welfare Chiang Mai, Thailand, pp 268.
- Kamperdick C, Bretmaier E, Radloff M (1992). A new steroid saponin from *Vernonia amygdalina* Del. (Compositae). J. Prakt. Chem. 334: 425-428.
- Kim H (1990). Comparison of antioxidant activity of α -carotene, lutein and lycopene by high pressure liquid chromatography. Korea J. Nutr. 23: 434-442.
- King RS, Teitel CH, Shaddock JG, Casciano DA, Kadlubar FF (1999). Detoxification of carcinogenic aromatic and heterocyclic amines by enzymatic reduction of the N-hydroxy derivative. Cancer Lett. 143: 167-171.
- Kingston DGI (1992). Taxol and other anticancer agents from plants. In J.D Coombes, ed., IBC Technical Services Ltd., London New Drugs from Natural Sources. Pp. 101-119.
- Kupchan SM, Hemingway RJ, Karim A, Werner D (1969). Tumor inhibitors. XLVII. Vernodaline and vernomygdin, two new cytotoxic sesquiterpene lactones from *vernonia amygdalina* del. J. Org. Chem. 34: 3908-3911.
- Kwon-Ndung EH, Misari SM (2002). Agricultural biotechnology and poverty alleviation in Nigeria In Proceedings of the International conference on Biotechnology: commercialization and food security. Eds Ogbadu GH, Onyenekwe PC, pp. 123-132.
- Lamkanra A, Ogundani AO, Ogungbamila FO (1990). Antibacterial constituents of *Alchornea cordifolia* leaves Phytotherapy Res. 4: 98-100.
- Loder W (1966). The structure of methoxyellipticine. Aust. J. Chem. 19: 1947-1950.
- MacFarlane N, Swetmen AA, Coursey DG (1984). Comparison of traditional and industrial palm oil. Palm oil News 28: 11-17.
- Maillard LC (1912). Action des acides amines sur les sucres. Formation de melanoidines par voie methodique. Comptes Rendus Hebdamadaires des Seances de l' Academie des Sciences. 154: 66-71.
- Mandlekar S, Kong AN (2001). Mechanisms for tamoxifen. Apoptosis 6: 469- 477.
- Manorama R, Rukmini C (1991). Nutritional evaluation of crude palm oil in rats. Am. J. Clin. Nutr. 53: 1031S-1033S.
- Mantel SH, Haque SQ, Whitehall AP (1980). Apical meristem tip culture for the eradication of flexuous rod viruses in yams (*Dioscorea alata*). Tropical Pest Management 26: 170-179.
- Martin FW, Ruberte R (1975). The polyphenol of *D. alata* (yam) tubers associated with oxidative browning. J. Agric. Food Chem. 24: 67-70.
- Misra N, Misra P, Dutta SK, Mehrotra S (2003). A new method for micro propagation of Indian Sarsaparilla-*Hemidesmus indicus* RBr. embryogenesis Abstract, 3rd World Congress on Medicinal and aromatic plants for human welfare Chiang Mai, Thailand, pp 278.
- Nakamura Y, Hidaka M, Masaki H, Seto H, Udzumi T (1990). Major anthocyanin of flower of Hibiscus Sabdariffa. Agric. Biol. Chem. 54: 3345-3351.
- Ng JH, Tan B, (1988). Analysis of palm oil carotenoids by HPLC with diode-array detection. J. Chromatographic Sci. 26: 463-469.
- Njoku E (1963). The propagation of yams (*Dioscorea species*) by vine cuttings. J. West Afr. Sci. Assoc. 8: 29-32.
- Nwankwo JO, Tahnteng JG, Emerole GO (2000). Inhibition of aflatoxin B₁ genotoxicity in human liver-derived HepG2 cells by kolaviron biflavonoids and molecular mechanisms of action. Eur. J. Cancer Prev. 9: 351- 361.
- Ogundipe OO, Moody JO, Houghton PJ, Odelola HA (2001). Bioactive chemical constituents from *Alchornea laxiflora* (benth) pax and hoffman. J. Ethnopharmacol. 74: 275-280.
- Ogundipe OO, Moody JO, Odelola HA (1999). Biological activities of *Alchornea laxiflora* extractives. In: proceedings of the 1st International Workshop on the Standardization and Utilization of herbal Medicines: Challenges of the 21st Century, University of Ibadan, pp 201-208.
- Ohigashi H, Jisaka M, Takagaki T, Nozaki H, Tada T, Huffman MA, Nishida T, Kaji M, Koshimizu K (1991). Bitter principle and a related

- steroid glucoside for *vernonia amygdalina*, a possible medicinal plant for wild chimpanzees. *Agric. Biol. Chem.* 65: 1201-1203.
- Okezie CEA, Nwoke FIO Okonkwo SNC (1986). Field studies on the growth pattern of *Dioscorea rotundata* Poir propagated by seed. *Tropical Agriculture (Trinidad)* 63: 22-24.
- Okonkwo CEA, Nwoke FIO, Njoku E (1986). Pattern of growth of whole plants and development of vine nodal cuttings of *Dioscorea bulbifera* L. *Proc. Nig. Acad. Sci.* 1: 30-38.
- OMS, UICN, WWF (1993). Directrices sobre conservacion de plantas medicinales pp 55 UICN Publication, Gland.
- Ozo O N (1985). Analytical procedure for the polyphenols and polyphenol oxidase of yam tubers. In *Advances in yam Res. ed.* Godson Osuji, pp. 165-212.
- Phillipson JD, Wright CW, Kirby GC, Warhust DC (1993). Phytochemistry of some plants used in traditional medicine for the treatment of protozoal diseases. Abstracts, International Symposium of the Phytochemical Society of Europe; University of Lausanne, Switzerland, p L3.
- Rabehault H, Martin JP, gas S (1972). Recherches sur la culture des rissue de palmier a huile (*Elaeis guineensis* Jacq). *Oleagineux* 27: 531-534
- Reddy MK, Rao AS, Rao MV (2003). In vitro multiplication of *Tinospora cordifolia* Miers, an Ayurvedic Medicinal plant, via somatic embryogenesis Abstract, 3rd World Congress on Medicinal and aromatic plants for human welfare Chiang Mai, Thailand, pp 267.
- Resendiz-Lopez I, Loara-pina B, Castano-Tostado E (1998). Antimutagenicity of natural phenolic compounds in dried flowers from *Hibiscus sabdariffa* L against 1-nitropyrene. On line: File :// A:\Hib 5. htm.
- Roja G, Rao PS (2000). Anticancer compounds from tissue cultures of medicinal plant. *J Herbs, Spices. Med. Plants* 7: 71-102.
- Sarumi MB (2002). A peep into the activities of the National center for genetic resources and biotechnology (NACGRAB) and the relationship with the International center for genetic engineering and biotechnology (ICGEB). In *Proceedings of the International conference on Biotechnology: commercialization and food security.* Eds Ogbadu GH, Onyenekwe PC, pp. 117-122.
- Slater TF (1984). Free radical mechanisms in tissue injury. *Biochem. J.* 222: 1-5.
- Sonaiya EB, Omitogun OG (2002). Regional and International cooperation for sustainable agrobiotechnology in Africa In *Proceedings of the International conference on Biotechnology: commercialization and food security.* Eds Ogbadu GH, Onyenekwe PC, pp. 105-116.
- Stufness M, Douros J (1982). Current status of the NCI plant and animal product program. *J. Nat. Prod.* 45: 1-14.
- Tan, B and Chu, F.L (1991). Effects of palm oil carotenoids in rat hepatic cytochrome P450-mediated benzo(a)pyrene metabolism. *Am. J. Clin. Nutr.* 53: 1071S- 1075S.
- Tanaka T, Kawamori T, Ohnishi M, Okamoto K, Mori H, Hara A (1994). Chemoprevention of 4-nitroquinoline 1-oxide-induced oral carcinogenesis by dietary PCA during initiation phase. *Cancer Res.* 54: 2359-2365.
- Tanaka T, Kojima T, Kawamori T, Mori H (1995). Chemoprevention of digestive organ carcinogenesis by natural product protocatechuic acid. *Cancer Suppl.* 75: 1433-1439.
- Tanaka T, Kojima T, Kawamori T, Yoshimi M, Mori H, (1993). Chemoprevention of deithylnitrodamine-induced hepatocarcinogenesis by a simple phenolic acid, protocatechuic acid in rats *Cancer Res.* 53: 2775-2779.
- Terao J (1989). Antioxidant activity of β -carotene-related carotenoids in solution. *Lipids* 24: 659-661.
- Terashima K, Aqil M, Niwa M (1995). A novel biflavonoids from the roots of *Garcinia kola*. *Heterocycles* 41: 2245-2250.
- Terashima K, Kondo Y, Aqil M, Waziri M (1999). A study of biflavanones from the stem of *Garcinia kola* (Gutiferae). *Heterocycles* 50: 238-90.
- Terashima K, Takawa Y, Niwa M (2002). Powerful antioxidative agents based on Garcinoic acid from *Garcinia kola*. *Bioorg. Med. Chem.* 10: 1619- 1625.
- Tseng TH, Kao ES, Chu HY, Chou FP, Linwu HW, Wang CJ (1997). Protective effects of dried flower extracts of *Hibiscus sabdariffa* L against oxidative stress ion rat primary hepatocytes. *Food Chem. Toxicol.* 35: 1159-1164.
- Tseng TH, Wang CJ, Kao ES, Chu HY (1996). Hibiscus protocatechuic acid protects against oxidative damage induced by *tert*-butyl hydroperoxide in rat primary hepatocytes. *Chem. Biol. Int.* 101: 137-148.
- Tsuda T, Shiga K, Ohshima K, Kawakish S, Osaw T (1990). Inhibition of lipid peroxidation and the active oxygen radical scavenging of anthocyanin pigments isolated from *Phascolius vulgaris* l. *Biochem. Pharmacol.* 53: 1033-1039.
- Vasanthakumar K (1981). Studies on the propagation and growth of sapogenin-bearing yams, *Dioscorea floribunda* Mart and Gal, and *Dioscorea composita* items from single leaf stem cuttings Thesis Abstracts 7: 69-70.
- Walker JRL (1975). Enzymic browning in foods. *Enz. Technol. Dig.* 4: 89-100.
- Wan MC, Taylor HL, Wall ME, Coggon P, McPhail AT (1971). Plant antitumor agents VI. The isolation and structure of Taxol, a novel anti leukemic and antitumor agents from *Taxus brevifolia*. *J. Am. Chem. Soc.* 93: 2325-2327.
- Wang CJ, Wang JM, Lin WL, Chu CY, Tseng TH (2000). Protective effect of Hibiscus anthocyanins against *tert*-butyl hydroperoxide-induced hepatic toxicity in rats. *Food Chem. Toxicol.* 38: 411-416.
- Wogan G.N, Paglialunga S, Newbwerne PM (1974). Carcinogenic effects of low levels of aflatoxin B1 in rats. *Food Cosm. Toxicol.* 12: 681-685.
- Woodall AA (1994). Carotenoids and the protection of membrane against oxidative damage. Ph.D thesis. University of Liverpool, Liverpool, U.K.
- Woodall AA, Lee SW, Weesie RJ, Jackson MJ, Britton G. (1997a). Oxidation of carotenoids by free radicals: relationship between structure and reactivity. *Biochim. Biophys. Acta* 1336: 33-42.
- Woodall AA, Britton G, Jackson MJ (1997b). Carotenoids and protection of phospholipids in solution or in liposomes against oxidation by peroxy radicals: Relationship between carotenoid structure and protective ability. *Biochim. Biophys. Acta* 1336:575-586.