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Research Article

IN SEARCH OF NEW LEADS: A CLOSER LOOK AT THE THERAPEUTIC POTENTIAL OF THE CONSTITUENTS OF *MILLETTIA THONNINGII, MILLETTIA PACHYCARPA* AND THEIR STRUCTURAL ANALOGUES

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ABSTRACT

This review discusses the potential applications of isoflavonoids from *Millettia thonningii* and *M. pachycarpa* in developing new pharmaceutical agents based on folkloric anecdotes and evidences from pharmacological and biochemical assays and to encourage further research into their pharmacological applications. *Millettia thonningii* is a deciduous plant indigenous to tropical West Africa, *M. pachycarpa*, is a climbing shrub indigenous to South-East Asia and other species have been used in folk-medicine for the treatment of inflammatory diseases, chronic diseases and several pathogenic diseases. Scientific research has implicated several prenylated isoflavonoids as being useful antioxidants and used in the management of radical-mediated diseases such as cancer, diabetes, ischemic heart diseases, Alzheimer's and Parkinson's diseases etc. Chemical investigations into these plants have revealed that they contain several isoflavonoids which bear structural resemblance to some of the isoflavonoids already in allopathic medical usage. Plants continue to maintain their historical stead as a store house of important drug candidates and source of new "leads" for synthetic modifications to improve activity through optimization of pharmacodynamic and pharmacokinetic properties. Natural products have also been found useful in specific pharmacological probes, a potential which is grossly underestimated. The alpinumisoflavones which have been isolated from the *Millettia thonningii* have shown high toxicities to the brine shrimp while isolates from *M. pachycarpa* have also shown anti-estrogenic and anticancer properties. Information obtained from crystal structural studies of these alpinumisoflavonoids coupled with their molecular and electronic distribution properties can further our understanding of their therapeutic potential and their observed bioactivities. The alpinumisoflavones are characterized by a fused tricyclic ring system which contains nearly coplanar benzopyrone ring fragments and a puckered six membered pyran ring that adopts a half-chair conformation with inter and intra molecular O-H….O and C-H…O contacts. A phenyl ring attached to the benzopyrone moiety shows out of plane twist with various degrees of torsion depending on the substitution on the phenyl ring.

Keywords: *Millettia thonningii, M. pachycarpa,* Isoflavones, Folk-medicine, crystal structure, flavonoids, Alpinumisoflavones.

INTRODUCTION

Millettia thonningii (Schum-Thonn) Baker is a deciduous tree which grows in tropical climates all over the world. It belongs to the family Papilionaceae and is indigenous to tropical West Africa and is found in moist areas and occasionally arid savanna regions[1]. It is among the commonest of close to 150 plants in this genus scattered all over the world. It can grow as tall as 20m with green or grey bark and pinnately shaped leaves which are arranged alternately on the branches. Flowering of the plant normally starts around September while the fruits start appearing in December. Both seed dispersal and vegetative propagation are used as means of propagation[2].

M. pachycarpa Benth (*M. taiwaniana*) is a deciduous climbing shrub indigenous to South-East Asian tropical forests. It belongs to the family Fabaceae and can grow to a height of about 6m. It has large clusters pea-shaped flowers that are lilac coloured. The stem is usually brown or grey with dark brown seeds. The flowering usually occurs in July and August[3].

Ethnobotanical uses of *Millettia* species

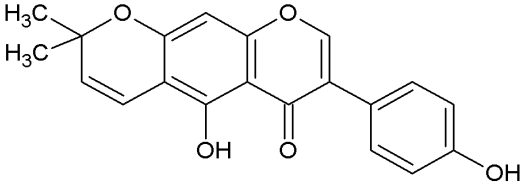
Millettia thonningii has been used in traditional medical practice in alleviating several ailments. According to folkloric anecdotes, Francophone countries in West Africa use a bark infusion to treat constipation in children[4]. In Nigeria however, it is reported that the leaf extracts of this plant cure diarrheal symptoms as well as dysentery. The pulverized roots and bark decoction are reported to relief symptomatic episodes of dysmenorrhea and amenorrhea as well as analgesic against intestinal pains. It is also drunk as a "blood purifier" and as a de-wormer. The leaf juice is said to be lethal to the Bulinus snail, a water snail which is a vector for Schistosoma cercariae which causes schistomiasis, a parasitic disease endemic in Africa, Far East and South America[4,5].

Milletia oblate Dunn is used to treat bladder troubles with the root decoction used as a cure for cough and stomach ache. *Millettia lasiantha* is used as an aphrodisiac either by chewing the roots or drinking aqueous decoction of it while gargling root extracts of *Milletia makodensis* Harms gives relief from toothache[6].

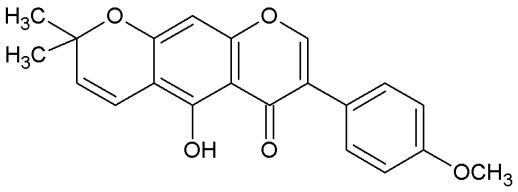
Roots extracts of *Millettia pachycarpa* Benth are used in China for fishing by pouring the root extracts in the water body which poisons and kills them. Saponins and rotenone related compounds have been implicated in this activity[7]. Elsewhere, extracts of *Millettia barteri* Dunn are used for this purpose while the dried and pulverized bark of the tree is used as a snuff against sinusitis and headaches[8]. In India, *Millettia auriculata* is used as an effective insecticide[9] as well as applied to cattle sores to kill vermin. Seeds of *Millettia ferruginea* Baker however are used as vermifuge against roundworms[10].

Isolated compounds from various parts of *Millettia thonningii*

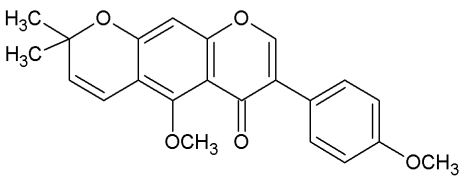
Several compounds have been isolated from several parts of the plant. The following are some examples.



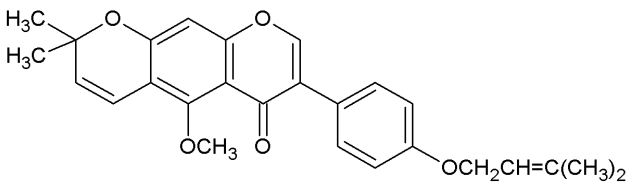
Alpinumisoflavone [1]



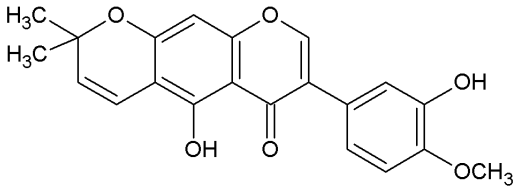
4'-O-methylalpinumisoflavone [2]



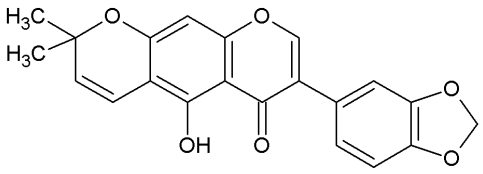
O,O-dimethylalpinumisoflavone [3]



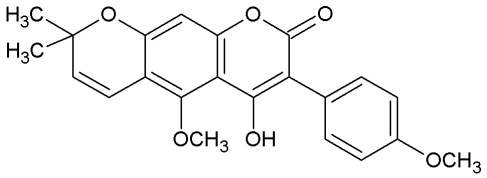
5-O-methyl-4-O-(3-methylbut-2-en-1-yl)alpinumisoflavone [4]



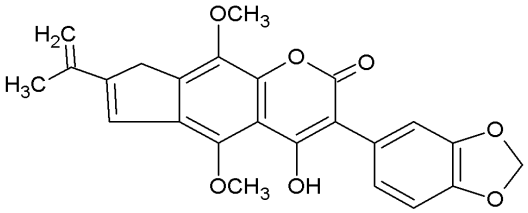
3',5-dihydroxy-4-methoxy-2",2"-dimethylpyrano(5",6",7)-isoflavone [5]



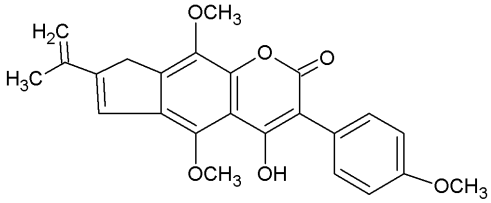
Robustone [6]



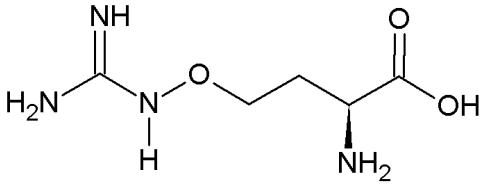
Robustic acid [7]



Thonningine-A [8]

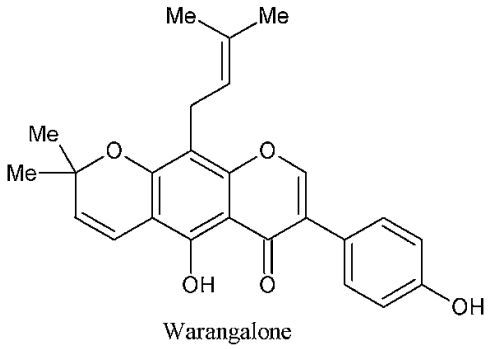


Thonningine - B [9]

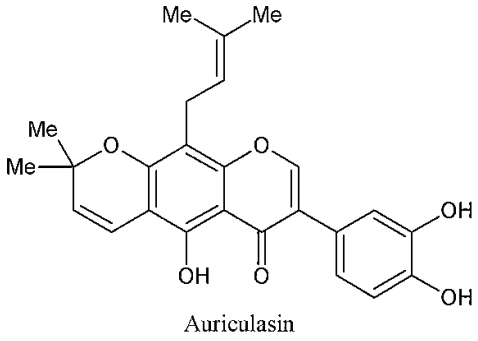


Canavanine [10]

Fig. 1: Some isolated compounds from *Millettia thonningii*.



Warangalone



Auriculasin

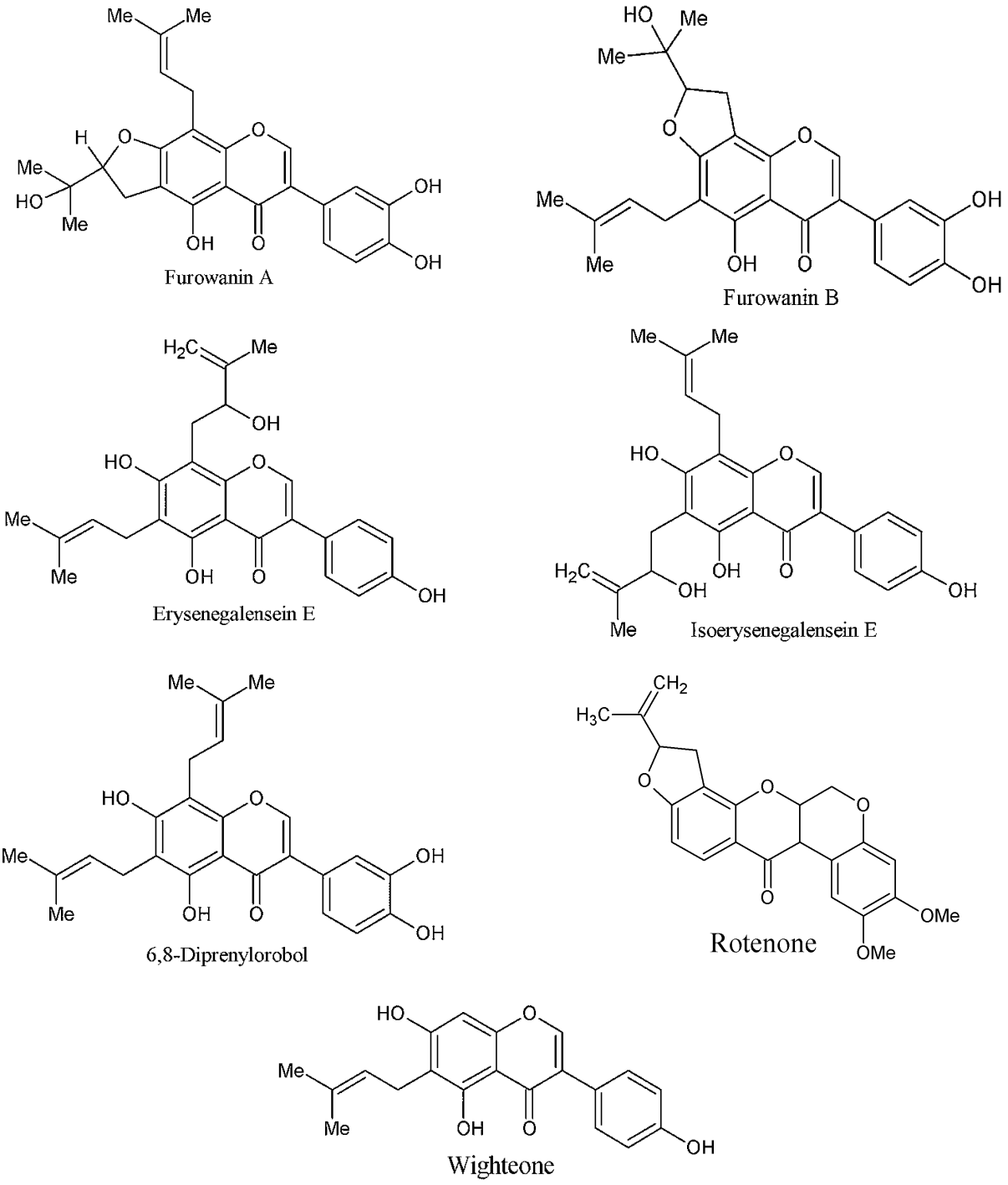


Fig. 2: Some isolated compounds from *Millettia pachycarpa.*

Some compounds isolated from *M. pachycarpa.*

The above are some of the compounds isolated from *M. pachycarpa* also known as *M. taiwaniana.* Compounds with similar structural backbone have been isolated from other species of *Millettia*.

Biosynthesis of Flavonoids

Alpinumisoflavones share ancestry with a general class of compounds called flavonoids which are a group of over 8000 polyphenolic compounds that occur exclusively but with very wide distribution in plant species. They are characterized by a phenylbenzopyrone structure (C6-C3-C6), and their categorization is based on the level of saturation and opening of the central pyran ring, into: flavones, flavanols, isoflavones, flavonols, flavanones, and flavanonols. They are produced biogenetically from two main primary synthetic pathways- the acetic acid pathway and the shikimate pathway which produces phenylalanine and tyrosine[11]. The flavonoids are formed in plants and participate in the light-dependent phase of photosynthesis during which they catalyze electron transport[12]. Phenylalanine and tyrosine are converted to cinnamic acid and parahydroxycinnamic acid, respectively, by the action of phenylalanine and tyrosine ammonia lyases[13]. The rest of the biosynthetic pathway is summarized below.

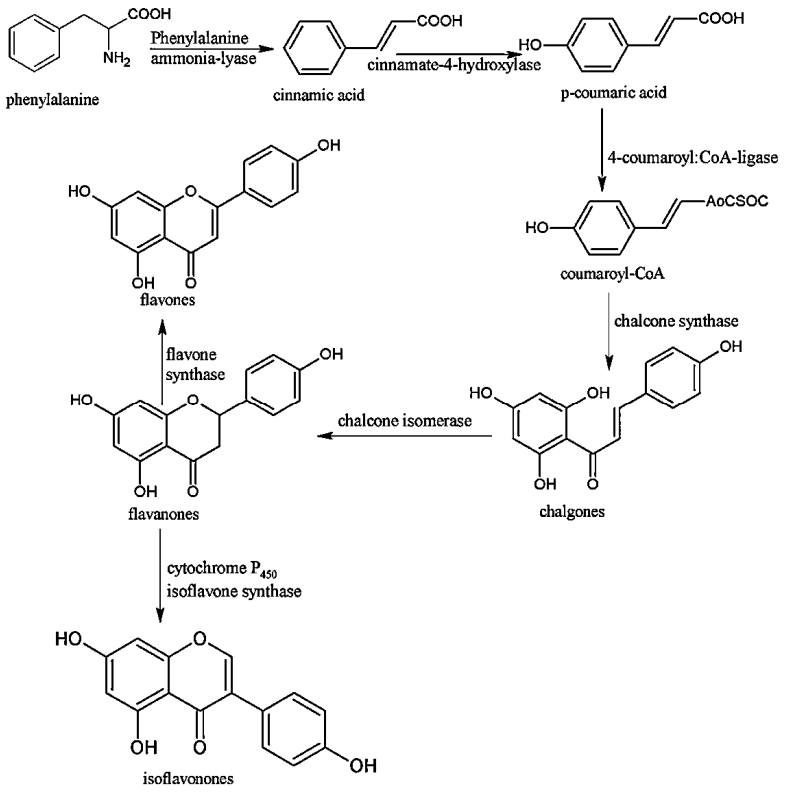


Fig. 3: Biosynthetic route to flavonoids

Pharmacological profile of constituents of *Millettia thonningii* and *M. pachycarpa*

Over the years, scientific research continues to accrue evidences in support of the folkloric use of plants for curative purposes. Phytochemical research has led to isolation of several different compounds from plants, most of the time with entirely different bioactivities. These numerous compounds with myriad of bioactivities may somehow give credence to the use of one decoction of a plant in curing several different ailments in our traditional African herbal practices. Unfortunately however, many people who do not trust the therapeutic potential of herbal medicines question the use of one herb in curing a 'million' diseases without the benefit of a scientific evidence of the bioactive components to support or contradict these claims.

While the patronage of herbal medicines still remains very low among the elite, plants continue to maintain the historical stead as important source of new drug candidates for the management of several diseases and also as source of new 'leads' for synthetic modifications to optimize pharmacokinetic and pharmacodynamic properties. They may be useful for specific pharmacological and biochemical probes[14]. According to the WHO, 80% of the world population continues to rely mainly on traditional medicines for their health care[15].

Millettia thonningii and *M. pachycarpa* have been used in folk-medicine for the treatment of pains, pathogenic diseases and radical-mediated diseases for ages. In trying to ascertain the efficacy or otherwise of the constituents of these plants in justification of their folkloric use, several bioactivity tests have been conducted on some extracts of various parts of the plants. In addition several bioactivity tests have been carried out on extracts of some other plants which have constituents that are also found in *Millettia thonningii* and *M. pachycarpa.* The following chronicles some of the major biochemical assays carried out on some of the constituents of these plants.

Anti-estrogenic and anticancer activity

Hypoxia is a condition characterized by lack of oxygen in the tissues of the body of organisms and is concomitant with cancers and tumors when oxygen demand of the rapidly dividing cells are not met. In dealing with this situation, the body's feedback mechanisms cause hypoxic tumor cells to activate the transcription of genes that function to promote anaerobic metabolism as well as those that initiate tumor angiogenesis[16-18]. A common feature of this situation is that some regions of tumor cells are constantly hypoxic which causes them to become more aggressive and resistant to treatment[19] in their bid to adapt to the low oxygen and nutrients supply.

First described by Semenza and Wang[20] as transcriptional activator responsible for the hypoxic induction of erythropoietin and for that matter a key regulator of oxygen homeostasis, hypoxia-inducible factor-1 (HIF-1) a heterodimer of the basic helix-loop-helix PER-ARNT-Sim proteins has a basic structure made up of constitutively expressed HIF-1β subunit and a HIF-1α (HIF-1α and HIF-1β/ARNT) subunits[21] inhibition of which offers a major biochemical target for the discovery of hypoxia-selective anticancer drugs. HIF-1 has been found to regulate the expression of genes involved in processes such as immortalization, genetic instability, dedifferentiation and stem cell maintenance, tumor angiogenesis, metabolic reprogramming, survival and resistance to apoptosis, migration/invasion and metastasis, and treatment resistance. These it does by promoting hypoxic adaptation and survival by increasing oxygen delivery and decreasing oxygen consumption, expressing growth factors for autocrine signaling, suppressing cell death, and promoting metastasis[22, 23]. Research based on animal models suggest that, proliferating cells express vascular endothelial growth factor (VEGF) gene, which stimulates angiogenesis to provide the additional perfusion that is required to maintain oxygenation of an increased number of cells hence HIF-1 inhibition reduced tumor vascularity

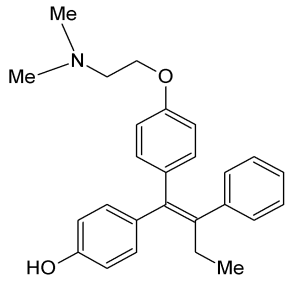
and retarded tumor growth hence HIF-1α protein expression is indicative of the advanced state of the disease, metastasis, treatment resistance, and poor prognosis in cancer patients [22, 24-28]. A combination of chemotherapy or radiation with HIF-1 has been found to produce enhanced treatment outcomes in preclinical studies [29, 30].

Liu Yang *et al*[31] using natural product chemistry based approach in the search for HIF-1 inhibitors with the conviction that small molecule HIF-1 inhibitors represent potential drug leads that will suppress tumor growth and enhance chemotherapy/radiation by inhibiting hypoxia-induced gene expression report after studying the lipid extract of *Lonchocarpus glabrescens* strong inhibition of HIF-1 by the constituents of this plant. Alpinumisoflavone and 4'-O-methylalpinumisoflavone which are copiously abundant in seeds and the root-bark of *Millettia thonningii* are the two compounds implicated in this bioactivity. In human breast tumor T47D cells, Alpinumisoflavone and 4'-O-methylalpinumisoflavone inhibited hypoxia-induced HIF-1 activation with IC_{50} values of 5 and 0.6 μM, respectively.

At the concentrations that inhibited HIF-1activation, 4'-O-methylalpinumisoflavone inhibited hypoxic induction of HIF-1 target genes (*CDKN1A*, *GLUT-1*, and *VEGF*), tumor angiogenesis *in vitro*, cell migration, and chemotaxis. 4'-O-methylalpinumisoflavone inhibits HIF-1 activation by blocking the induction of nuclear HIF-1α protein, the oxygen-regulated subunit that controls HIF-1 activity. Mechanistic studies indicate that, unlike rotenone and other mitochondrial inhibitors, 4'-O-methylalpinumisoflavone represents the first small molecule that inhibits HIF-1 activation by simultaneously suppressing mitochondrial respiration and disrupting protein translation *in vitro*. This unique mechanism distinguishes 4'-O-methylalpinumisoflavone from other small molecule HIF-1 inhibitors that are simple mitochondrial inhibitors or flavanoid-based protein kinase inhibitors.

When isoflavonoids isolated from the leaves of *Millettia pachycarpa* (*M. taiwaniana*) were tested for the inhibitory potential to the growth of human leukemia HL-60 cells, furowanin-A, Warangalone, Isoerysenegalensein-E, and euchrenone b_{10} showed the most significant cytotoxicity. Furowanin-A, Warangalone, and Isoerysenegalensein-E, were found to induce apoptosis in HL-60 cells through activation of the caspase-9/caspase-3 pathway, which is triggered by mitochondrial dysfunction[32].

Isoerysenegalensein E and 6, 8- diprenylorobol were found to be the most potent antagonists of the estrogen receptor (ER) with their anti-estrogenic activity comparable to that of 4-hydroxytamoxifen, a metabolite of tamoxifen and a stronger ER antagonist than tamoxifen. Warangalone and auriculasin were found to be weak ER inhibitors, and alpinumisoflavone and erysenegalensein E were non-ER inhibitors[33]. These finding has been corroborated by Ito *et al* [34].



4-hydroxytamoxifen (OHT)

Anti-infective properties

When 10g of powdered seeds of *Millettia pachycarpa (M. taiwaniana)* were extracted with 100ml of water, acetone and ethanol and applied as fine spray against the adult housefly, *Mussa domestica*, the fully grown larvae of the cabbage worm *Pieris rapae* and the silk worm *Bombyx mori*, the ethanol extract was found to be the most toxic against the cabbage worm, while the acetone extract was most effective against housefly. The aqueous extract was found to be toxic to the housefly, cabbage worm and Pareva larvae. Rotenone and rotenoid group of compounds have been established as the active constituents in these extracts[35].

Mukerja and Tripathi[36], reported that the ether extract of the seeds of *Millettia pachycarpa* was found to be one-third as toxic as dichlorodiphenyltrichloromethane (DDT) when used as contact poison for houseflies. At a concentration of 0.5%, the extract was 80-90% lethal to silkworm larvae while at 1% it was 100% lethal to the eggs of silkworm.

Bishnupada Roy *et al*[37], report that, crude ethanol, methanol, and acetone fractions of *M. pachycarpa* when assayed against *Raillietina echinobothrida*, the intestinal cestode parasite of domestic fowl, in an effort to verify the putative anthelmintic efficacy and cestocidal potential of this plant realized that *in vitro* exposure of the worm to the extracts at a concentration of 25 mg/mL in phosphate buffered saline (at 37°C ± 1°C) caused distortions and disruption of mitochondria, nucleus, nucleolus, nuclear membrane, basal lamina, and tegumental vacuolization in the distal cytoplasm leading to scar formation in the surface.

Perrett *et al* [38], investigating the potential anthelmintic properties of *Millettia thonningii*, found that a chloroform extract of the seeds when topically applied to mouse skin prior to exposure to *Schistosoma mansoni* cercariae, showed molluscicidal and cercaricidal activity. Subsequent re-infection was inhibited for as long as the extracts of *Millettia thonningii* were present on the surface of the animal skin perhaps justifying the use of the plant as an anthelmintic and a purgative according to folklore[4,5]. The compounds implicated for this activity are thought to be the isoflavonoid alpinumisoflavone and its derivatives. In furtherance of the work done above, *in vitro* bioactivity study by Laddiard *et al* [39], and later corroborated by Maillard *et al* [40], shows that the extracts of the seeds of this plant are lethal to *Schistosoma mansoni* miracedia, cercariae, and adult worms. Robustic acid and other coumarin compounds present in the seeds and alpinumisoflavones are said to be responsible for this observed bioactivity.

In investigating the possible mode of this anti-schistosomal bioactivity using rat liver as the test organ, Laddiard *et al* [39,41], found that the seed extracts of *Millettia thonningii* inhibited the site I mitochondrial electron transport system's NADH dehydrogenase at concentrations of 30-159mg/l. While these bioactivities have been unambiguously established, it is not clear which of the many isoflavones are responsible for these bioactivities and how the substitution pattern and their resulting molecular structures influence these bioactivities.

Monoamineoxidase enzyme inhibition

The surge in Parkinson's and Alzheimer's diseases which are aging-related neurodegenerative diseases has increased immensely the interest in the search for compounds which have this therapeutic potential. It has been observed that selegiline, rasagiline and lazabemide, which are inhibitors of Monoamine oxidase- B implicated in these neurodegenerative diseases have also been observed to have protective effects on neuronal tissues increasing the interest in compounds which have this therapeutic potential[42]. In assessing the therapeutic potential of the fruits of *Cudrania tricuspidata*, it was found that they contain prenylated flavones which were found to be potent Monoamine oxidase-A&B (MAO-A/B) inhibitors in concentration dependent manner and could be possible therapeutic candidates for the Parkinson's and Alzheimer's diseases. However further pharmacological investigations and *in vivo* physiological functions remain unelucidated. Some of the compounds found to be responsible for this (MAO-A/B) inhibitory effects included 4'-O-methylalpinumisoflavone and alpinumisoflavone[43], which are copiously abundant in *Millettia thonningii*. The slight differences in IC_{50} values 23.9, and 25.8 μM, respectively found for 4'-O-methylalpinumisoflavone and alpinumisoflavone makes it imperative for other compounds in this plant to be investigated.

Allelopathic uses

In search of potential herbicides, phytotoxic effects of the extracts of

the aerial part of *Pueraria phaseoloides* were evaluated using Petri dish bioassay on the potential inhibition of the seed germination of the weed species of *Mimosa pudica, Senna obtusifolia, Senna occidentalis and Urena lobata*. Arruda *et al* [44], report that, two of the compounds isolated from this plant and used for the bioassay are 4'-O-methylalpinumisoflavone and alpinumisoflavone. The inhibition on germination on the various weeds was found to be more selective. At 3ppm, the inhibition of 4'-O-methylalpinumisoflavone on *S. obtusifolia* was found to be the strongest at 77% while alpinumisoflavone inhibited *U. obata* at 60%, highest observed in the experiment.

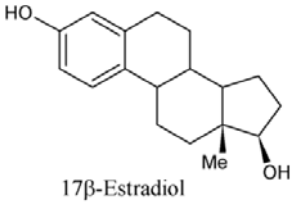
Pharmacological profile of structural analogues of the constituents of *Millettia thonningii* and *Millettia pachycarpa*

The constituents of *Millettia thonningii* share a lot of structural semblance with some very well-known flavonoids such as the catechins, epicatechins, genistein, daidzein, quercetin, Licopyranocoumarin, Glycycoumarin, Glycherrisoflavone, kaempferol, Glabrene, silybin and many others with well documented pharmacological profile. The structural diversity and its ubiquitous distribution in plant species give some credence to the assertion that flavonoids may have existed in nature for over one billion years affording interactions with evolving organisms over the eons and undergoing structural transformations sometimes without losing its precursor. If the theory of evolution is anything to go by, then flavonoids must possess some important functions in nature which have been critical to the plants to have been retained in vascular plants throughout evolution[45]. Indeed this long existence of flavonoids also means a long association with various animal species that have come through the evolutionary ladder and perhaps may be a justification for their array of biochemical and pharmacological activities in living systems.

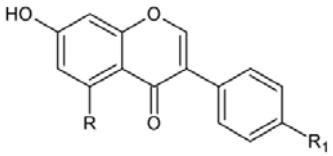
Flavonoid compounds undoubtedly have critical effects in the physiological and biochemical systems of plants imputed with functions such as antioxidants, enzyme inhibitors, precursors of toxic substances, pigments and light screens[46]. Furthermore they play active roles in photosensitization and energy transfer, the actions of plant growth hormones and growth regulators, the control of respiration, photosynthesis, morphogenesis, and sex determination, as well as defense against infection[47].

Estrogenic and antitumor bioactivity

A phenolic ring and a distance of 11.5Å between two hydroxyl groups in genistein are some structural features it shares with the most potent of the endogenous estrogen steroids, 17β- estradiol. These seemingly trivial features enable genistein to exert both estrogenic and anti-estrogenic activity by binding competitively to estrogen receptors and sex hormone binding proteins. Equol which is a non-steroidal estrogen produced as a metabolic product of isoflavonoids such as daidzein by gastrointestinal bacterial flora has proven to have beneficial effects in incidences of prostate cancer and some physiological changes accompanying menopause and together with genistein, are able to dislodge bound estrogen and testosterone from human sex steroid binding proteins resulting in their delayed clearance and hence availing the hormones to target cells[48-50].



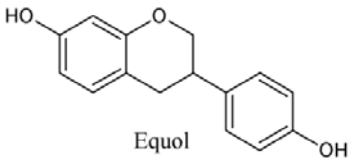
17β-Estradiol



Genistein : R= OH, R_1= OH

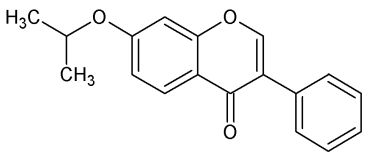
Daidzein : R = H, R_1= OH

Biochanin A : R= OH, R_1= OMe



Equol

Neonatal administration of genistein has been found to confer protection against chemically-induced mammary tumors in rats by inducing increased latency, reduced tumor incidence and multiplicity, and more rapid maturation of undifferentiated end buds to differentiated lobules[51]. Biochanin A (4'-methoxygenistein) copiously abundant in chickpea on the other hand, is an active cancer chemopreventant in animal models. The mechanism of inhibition of these isoflavones in breast cancers, cell growths and the development of chemically induced cancers in the stomach, bladder, lung, prostate, and blood involves stimulation of a signal transduction pathway leading to apoptosis[52]. Genistein blocks Epidermal Growth Factor (EGF) mediated tyrosine phosphorylation *in vivo* in human epidermal carcinoma cells but appears neither to induce phosphorylation of EGF receptors nor other tyrosine kinase substrates. It is suggested hence that the inhibition of cell growth is through modulation of transforming growth factor (TGF) β1 signaling pathways.



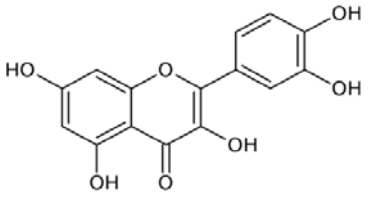
Ipriflavone

The development of ipriflavone as an oral treatment for acute leukemias and osteoporosis by increasing bone calcium retention, inhibition of bone breakdown, promotion of the activity of bone-building cells and reduction in the pain of osteoporotic fractures was based on the observation that genistein when targeted specifically to leukemia cell lines via a linkage to a monoclonal antibody, was found to be a strong inhibitor of the growth of the leukemia cells by selectively inhibiting CD-19-associated tyrosine kinase activities, resulting in death of human B-cell precursor leukemia cells[53]. However, in several cell systems in which genistein inhibits growth, the therapeutic window (index) has been found to be very wide and only exerts toxicity at concentrations greatly in excess of those at which it first exerts its biological and pharmacological effects making it a potentially important molecule for dietary cancer chemoprevention.

Protein kinases mediate phosphorylation of amino acid residues in proteins resulting in subsequent changes in the conformational identity of the protein and are found to be responsible for controlling several cellular functions. Protein tyrosine kinases (PTK) found in many different types of cells are implicated in focal adhesions which are perfunctory links to the extracellular matrix within which biochemical signaling of proteins at sites of integrin binding and clustering takes place invariably regulating cell transformation and growth, expression of genes, cell-cell adhesion interactions, cell motility, and shape[54-56]. Genistein was not only found to selectively inhibit PTK and at higher concentrations protein histidine kinase (PHK), but also has the propensity to inhibit DNA topoisomerases I and II which introduce transient breaks in linear DNA sequences and participate in several genetically related processes, including replication, transcription, recombination, integration, and transposition[57]. They are also found to possess antioxidant and cell cycle inhibitory activity[58].

Apart from its inhibitory effects on PTK and Protein Kinase C (PKC) activity, quercetin has been found capable of inhibiting nuclear kinase II-catalyzed phosphorylation of isolated nuclear proteins in

HeLa cells using Guanidine Tri Phosphate (GTP) as phosphate donor[59].

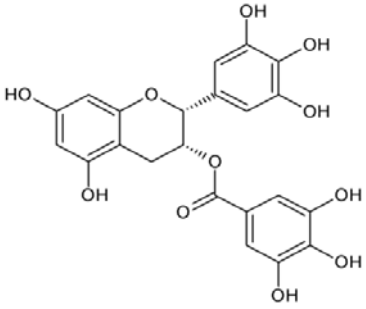


Quercetin

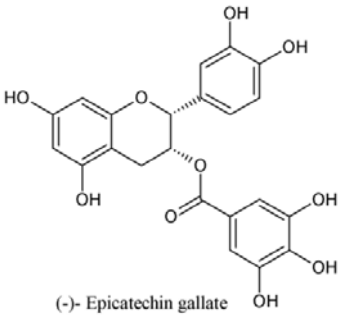
Anti-retroviral activity

Viruses continue to be a continuous source of worry for mankind being responsible for deadly diseases such as H1N1, Ebola, AIDS, some cancers and many other diseases. Retroviruses are Ribonucleic acid (RNA) viruses that copy their genomes into Deoxyribonucleic acid (DNA) during their replication. Reverse transcriptase (RT) enzymes transcribe the infecting RNA chains of these viruses into complementary DNA molecules that integrate into the host cell genome and this could lead to a permanent genetic alteration. Thus RTs have the potential to copy any RNA into DNA, even in the absence of specific transfer RNA (tRNA) primers such as Human T-lymphotropic viruses 1& 2, Murine leukemia virus, Human immunodeficiency virus (HIV) 1 &2 and Mouse mammary tumor virus. Encoded by the retrovirus RNA and packaged inside each viral capsid during the production of new virus particles, reverse transcriptase is described as an unusual DNA polymerase that utilizes either RNA or DNA as a template. Upon entry into a cell, the enzyme RT which accompanies the single-stranded RNA of the retrovirus makes a DNA copy of the RNA strand to form a DNA-RNA hybrid helix, which is then further transformed by the same enzyme to a DNA double helix. A virus-encoded integrase that catalyzes the insertion of the viral DNA into virtually any site on a host-cell chromosome recognizes the two ends of the linear viral DNA after which the integrated viral DNA is transcribed by host-cell RNA polymerase to produce numerous identical types. Enzymatic activities present in retrovirus are; RNA-dependent DNA polymerase (reverse transcriptase; RT), DNA-dependent DNA polymerase, Ribonuclease H (RNase H), Integrase and Protease[60].

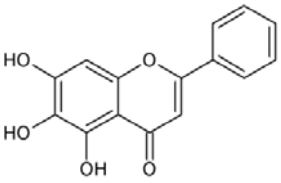
In their search for potential natural products that could inhibit key enzymes in the synthesis of DNA, Spedding *et al* [61], reported that quercetin was one of the three most active naturally occurring flavonoids that inhibited three reverse transcriptases (RT) viz: avian myeloblastosis RT, Rous-associated virus-2 RT, and Moloney murine leukemia virus (MMLV) RT when poly(rA)oligo(dT) 12–18 or rabbit globin messenger RNA (mRNA) were used as templates.



(-)- Epigallogocatechin gallate



(-)- Epicatechin gallate



Baicalein

Based on the observation that baicalein isolated from a Chinese traditional drug which is a flavone, inhibited HIV-reverse transcriptase (HIV-RT), a survey of the flavonoid family was carried out in which was found that (-)-epicatechin gallate (ECg) and (-)-epigallocatechin gallate (EGCg) which are major components of Japanese green tea strongly inhibited HIV-RT with IC_{50} values between 10-20ng/ml concentration[62,63].

Myricetin, morin, quercetin, and fisetin are some of the flavonoids suspected to be potential nonpeptidic inhibitors of the HIV protease enzyme with IC_{50} values in the range of 10 to 50 µM as reported by Brinkworth *et al* [64] while quercetin has been found to also strongly inhibit integrase activity[65].

Antioxidant activity

The evolutionary choice to use oxygen for respiration and oxygen containing compounds for diverse biological activities is not one without consequences. The ubiquitous presence of oxygen in the body of animals comes with it the production of other more "reactive oxygen species" (ROS) such as superoxide ($\cdot O_2^-$), hydroxyl radical ($\cdot OH$), peroxyl radical ($ROO\cdot$), alkoxyl radical ($RO\cdot$), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), triplet oxygen ($^3O_2$), hypochlorous acid (HOCl) and many others. The production of ROS in the body is essential for proper functioning but in excess, they pose several dangers to the health of individuals. When activated upon xenobiotic entry into the body of organisms, phagocytic cells such as monocytes, neutrophils, eosinophils, and macrophages generate $\cdot O_2^-$ as a result of increased oxygen consumption (respiratory burst), catalyzed by a membrane-bound NADPH oxidase system and are responsible for their bacteriocidal and tumoricidal functions[66,67].

While ROS generated by phagocytes play an important physiological function, they can also cause cellular damage to bio-systems which is a major contributor to degenerative diseases such as cancer, atherosclerosis, stroke, myocardial infarction, trauma, arthritis, ischemia/reoxygenation injury, and aging[68], peroxidation of membrane lipids, oxidative damage to nucleic acids and carbohydrates, and the oxidation of sulfhydryl and other susceptible groups in proteins[69,70].

In an attempt to ensure healthy life devoid of the dangers posed by ROS, defense to the body is provided by antioxidant systems which are involved in radical mob up by scavenging and quenching radicals hence detoxifying the body of ROS in the cell through enzymatic and non-enzymatic systems. The best known antioxidant molecules are vitamins A, E, and β-carotene, ascorbic acid, urate, ubiquinols, retinoids and carotenoids[71-73].

Many *in vitro* studies conducted on an array of compounds with the hope of finding potent radical scavengers found flavonoids as the most promising candidates, which contribute to the inhibition of lipid peroxidation and oxidation of low density lipoproteins (LDL) implicated in the pathogenesis of coronary heart diseases by decreasing the susceptibility of LDL to oxidation[74-76]. The protective effects of flavonoids in biological systems are ascribed to their capacity to transfer free radical electrons, chelate metal catalysts[77], activate antioxidant enzymes[78], reduce α-tocopherol radicals[79] and inhibit oxidases[80].

Consumption of food products rich in antioxidants has been amply demonstrated to confer a lot of health benefits. For example diets rich in rice, maize and beans have been shown to exhibit anticarcinogenic activity by inhibiting protease enzymes as well as interfering with the formation of ROS[81]. The agents present in these

diets to which are attributed these functions are flavonoids. Constituent of various types of teas which are basically flavonoids (catechins and theaflavins) have also been found to be anticarcinogenic and chemopreventive agents[82], radioprotective agents, antibacterial, antifungal and antiviral[83-86].

Crystal structure of Alpinumisoflavones and their bioactivity

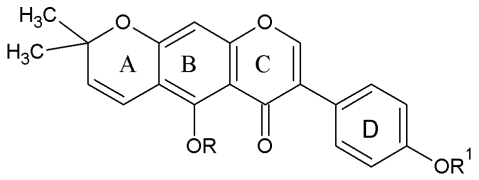
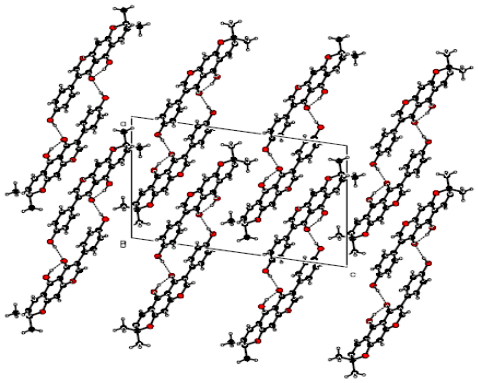


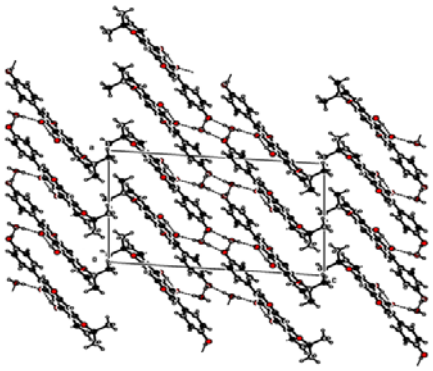
Fig. 4: General structure of the Alpinumisoflavones

The discovery of the electronic nature of drug-receptor interactions has opened the frontiers for crystal and bioactive engineering in a bid to improve predictability of bioactive compounds based on crystal structural studies. The electron-conformational method of pharmacophore identification and bioactivity prediction has seen great improvement in the usage of an atomic index of orbital and charge controlled interaction to better represent the ligand (substrate) in its interaction with the bioreceptor and the multi-conformational problem is considered in view of ligand–receptor binding states, resulting in essential simplification of the expression of bioactivity[87]. Systematic study hence of some of the compounds isolated from this plant, specifically the alpinumisoflavones (1-4) and their semi-synthetic analogues, have focused on crystal structure and activity[88-91], with the hope that the crystal structure, molecular and electronic properties can deepen our understanding of their observed bioactivities.

Not much diversity has been found among the crystal structures of the alpinumisoflavones studied so far. The tricyclic rings A/B/C are fused. The benzopyrone moieties consist of rings B and C of the compounds and are nearly coplanar. They show an out-of-plane twist with the phenyl ring D. However, the slight differences in substitution patterns have not only resulted in remarkable differences in bioactivities[89], but also produced some remarkably different crystal packing with various kinds of inter and intra-molecular hydrogen bonds some of which have been utilized in the preparation of solvates in a bid to improving its aqueous solubility properties to make bioassays *in vitro* even easier[91]. Electrostatic potential maps derived from non-spherical electron density using the Invariom method based on the approximation of locality of molecular electron density, compared to brine shrimp lethality tests conducted on some of the isolates, O,O-dimethylalpinumisoflavone, alpinumisoflavone and 5-O-Methyl-4'-O-(3-methyl-but-2-en-1-yl) alpinumisoflavone[89], indicates that the presence of high electron density around the O atoms of ring B and D are important for activity. Hence increasing the electron density in these areas by introducing more electron donating substituents R and R^1 without altering the conformations in these regions significantly may lead to higher activity *in vitro*. The following structures show some of diverse crystal structure packing found in the molecules so far studied.



Part of the crystal packing of alpinumisoflavone viewed on the *a-c* plane. Hydrogen bonds are shown as broken lines[90].



Part of the crystal packing of (alpinumisoflavone + H_2O) viewed along a-c plain showing the formation of molecular chains of fused rings. Hydrogen bonds are shown in broken lines[91].

Concluding remarks

The influence of lipid peroxidation in biological cells as being involved in the pathogenesis of many age-related diseases has long been known. Free radicals or Reactive Oxygen Species mainly produced by reactions such as abnormal oxidation and breakdown of fats and hydrogen peroxide have been implicated in the detoxification of invading organisms and chemical substances. Stray free radicals however, can also initiate lipid peroxidation in healthy cells, damage proteins and nucleic acids and other biomolecules through uncontrolled cleavages resulting in mutations that lead to chronic ailments. It has been observed that there is some positive link between accumulated free radical damage and some age-related diseases such as coronary heart diseases, diabetes, cancer etc and

neurodegenerative diseases such as Alzheimer's and Parkinson's[92,93]. Dietary flavonoids have been found to be able to repair a range of oxidative radical damage sustained by DNA with high intake of it having been associated with reduced risks to degenerative diseases[94].

Uncontrolled neo-vascularization leads to imbalances in the capillaries in the body and can promote angiogenesis dependent diseases such as growth of solid tumors, arthritis and inflammations making its inhibition important in the control of these diseases[95]. Plant extracts found to be rich in flavonoids have been found to possess high antioxidant and anti-angiogenic activities[96]. Perhaps the need to pay more attention to HIF-1 inhibitors has become more pertinent now not only because there is no approved drug that specifically targets hypoxic tumor cells but also as reported by Liu Yang *et al* [31] of the unique mechanism of the 4'-O-methylapinumisoflavone by simultaneously suppressing mitochondrial respiration and disrupting protein translation *in vitro*. The high inhibitory activity of 4'-O-methylalpinumisoflavone for HIF-1 was attributed by the authors to its high lipophilicity which increases its bioavailability. If this assertion is anything to go by, then the more lipophilic structural analogues of these compounds may perhaps be better candidates for this bioactivity. Constituents of *Millettia thonningii* and *M. pachycarpa* may be useful in this stead.

The discovery of monoamine oxidase inhibition potential of some constituents of this plant as already alluded to, make these plants potentially useful in the search for new drug candidates and "Lead" compounds for the synthesis of drugs to tackle the numerous radical mediated diseases. It must also be noted that anti-inflammatory effect as well as anti-microbial properties of these plants cannot be discounted having shown activity towards some pathological organisms such as worms. Utilization of the crystal structure information to model various receptor binding and docking experiments would further bring to light the therapeutic potentials of the constituents of these plants.

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