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Ochnaflavone, a Naturally Occuring Biflavonoid: Pharmacology and Prospects for Future Research

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript

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ABSTRACT

Aims: To profile and address matters surrounding ochnaflavone (**1**), derivatives and analogues.
Methodology: Papers concerning ochnaflavone, derivatives and analogues were accessed from search engines such as google scholar and pubmed.
Results: Ochnaflavone and derivatives are compounds bearing ether linkage between the two B-rings of the two flavonoid moieties. These compounds are known to possess potent biological activities such as anti-inflammatory, anticancer, anti HIV and antimicrobial.
Conclusion: In this paper, an overview summary of ochnaflavone, derivatives and analogues from past to present is presented. Thus, the paper provides a sense of focus to researchers; seal breaks the unattended matters, and therefore, spearheads new research areas concerning these compounds.

Keywords: Biflavonoid; ochnaflavone; pharmacokinetics; pharmacology; phytochemistry.

1. INTRODUCTION

A considerable attention has been directed to the use of herbal prescriptions in the form of medicines or supplements in recent years as a compensation for the perceived shortcomings of orthodox medicines [1]. The therapeutic effects of these herbal medicines could be attributed by

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the presence of phytochemicals, also known as secondary plant metabolites. These are naturally occurring compounds in plants specifically designed to protect plants from external hazards [2]. Most of these compounds in synergism or individually have proven to be beneficial to humans in medicinal aspects. Some of the attributes of phytochemicals that have given them approval by many societies include their easy availability and low cost. These compounds may act by causing hormone metabolism modulation, immune system stimulation, antineoplastic or decrease of platelet aggregation effects in the body [3].

Normally these compounds are not the essential nutrients for the body to sustain life but their ability to prevent and fight against diseases has been the focus of researches nowadays [4]. Among phytochemicals, those have recently attracted the attention of scientists worldwide, include flavonoids. This class of compounds is widely distributed among plants, especially in vegetables, fruits and many others [5]. Normally, these compounds are lower molecular weight polyphenolics and have been shown to possess many health benefits to humans [6-8].

Biflavonoids are represented by a small group of flavonoids that may generally be constituted by linking of two identical or non-identical units of flavones, flavanones, isoflavones, flavanols, chalcones, aurones and dihydrochalcones. The two units may be joined in a symmetrical or asymmetrical manner through the direct link between carbons of the two units (-C-C-) or through ether bond (-C-O-C-). The linkage between ring A of one flavonoid to ring B of another (AB biflavonoids) is the most commonly observed formation. Others constitute the linkage between the two A rings (AA biflavonoids) or between the two C rings (3,3"-CC biflavonoids). The most rarely observed biflavonoids from nature are the ones with a linkage between the two B rings (BB biflavonoids) [9].

The journey towards biflavonoids isolation began when ginkgetin [(**2**) as indicated in Fig. 1] was isolated from the leaves of maiden hair tree (*Ginkgo biloba* L.,) by Furukawa in 1929. However, to date, the numbers and diversity of biflavonoids keep on increasing [10,11]. This is due to the structural diversity arising from differences in the position and nature of interflavonoid linkage regardless of the substitution pattern [9]. The diversity among biflavonoids increases further with the presence and type of the substituent/functional groups and stereogenic centers in their skeleton [9]. So far, about 200 biflavonoids have been isolated from different Gymnosperm and Angiosperm species [12]. Few families such as Anacardiaceae, Berberidaceae, Burseraceae, Caprifoliaceae, Casuarinaceae, Euphorbiaceae, Guttiferae (especially Garcinia), Haemcdoraceae, Iridaceae, Labiatae, Leguminosae, Loganiaceae, Ochnaceae, Piperaceae, Rhamnaceae, Rutaceae, Salicaceae, Thymelaeaceae and Velloziaceae have been reported to be the sources of biflavonoids [13]. This is a clear indication that the pathway to which biflavonoids are biosynthesized is more specialized and takes place only in some few angiospermous families, thus, making these compounds of chemotaxonomic importance in some plant families such as Taxaceae and Iridaceae to Ginkgoaceae and Ochnaceae [14,15].

Biflavonoids have become the centre of attention to researchers these days, due to their ability to demonstrate diverse interesting biological activities caused by their structural variations. These compounds have shown an array of the pharmacological properties including antioxidant, antiproliferative, or anti-inflammatory, indicating their potentiality in pharmacological industry [16,17].

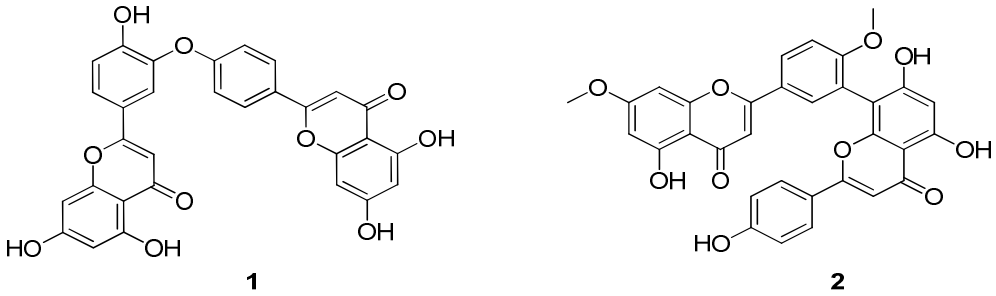


Fig. 1. Structures of ochnaflavone (1) and ginkgetin (2)

The biologically active ochnaflavone (**1**) and derivatives are BB′ biflavonoids that may either constitute flavone-flavone, flavone-flavanone or flavanone-flavanone units that are linked in C-O-C manner. The first isolation of compound **1** was reported from *Ochna squarrosa* Linn. (Ochnaceae) in 1973 by Okigawa et al. [18]. The compound and/or derivatives have since then been isolated from many other plant species especially those belonging to the family Ochnaceae, genus Ochna [19].

2. SOURCES

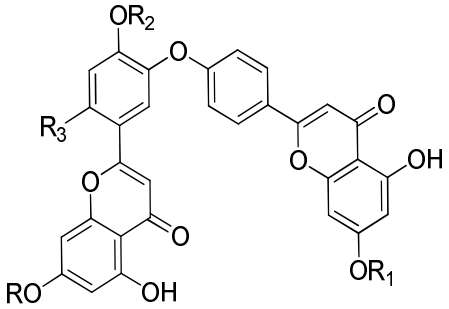
2.1 Natural Sources

So far, plants are the only natural sources of compound **1** and its derivatives. The following paragraphs describe the structures of the derivatives and their sources. The plant families such as, Ochnaceae, Caprifoliaceae, Paracryphiaceae (Quintiniaceae), Grossulariaceae and Selaginellaceae (club mosses or spike mosses) have been identified to be the sources of these compounds [13].

The isolation of ochnaflavone (**1**) has been reported from various plant species such as *Lonicera japonica* Thunb. (Leaves) [20] *Cespedesia macrophylla* Seem. (Leaves), *Cespedesia spathulata* (Ruiz & Pav.) Planch. (Leaves) [21], *Luxemburgia nobilis* Eichl. (Aerial parts) [22]. *Ochna beddomei* Gamble. (Leaves and stem bark) [23], *Ochna integerrima* (Louri) Merr. (leaves) [24], *Ochna lanceolata* Spreng. (Stem bark) [25], *Ochna obtusata* DC (Leaves) [26] and *Ouratea staudtii* Van Tiegh. Ex Keay. (Aerial parts) [27].

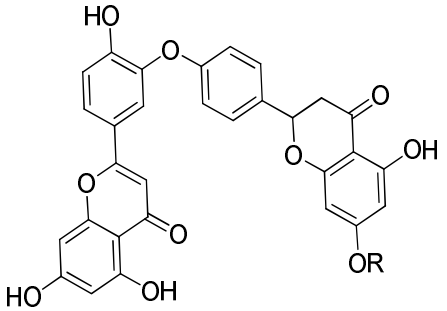
Derivatives of compound **1** as shown in Fig. 2, among others, 7″-*O*-methylochnaflavone (**3**) from the leaves of both *Cespedesia macrophylla* Seem. and *Cespedesia spathulata* (Ruiz & Pav.) Planch.,have been isolated [21]. The compound has also been reported from the leaves of *Ochna integerrima* [28]. 2′-hydroxy-4′-*O*-methyl derivative of this compound, triclisinone (**4**) has been reported from aerial parts of *Triclisia gilletii* [29].

The dihydro derivatives of compound **1**, such as 2",3"-Dihydroochnaflavone (**5**) have been obtained from leaves of *Luxemburgia nobilis* Eichl. [22] and aerial parts of *Luxemburgia octandra* St. Hil. [30]. The Isolation of the methoxy derivative of dihydroochnaflavones, viz. 7"-*O*-methyl-2",3"-dihydroochnaflavone (**6**), has been reported from the leaves of *Ochna integerrima* (Louri) Merr. [28]. 2,3-dihydroochnaflavone (**7**) was derived from the leaves of *O. integerrima* (Louri) Merr. [28], *Luxemburgia nobilis* [22] and *Ochna obtusata* DC. [26], the stem bark of *Ochna beddomei* Gamble. [23,26] and *Ochna lanceolata* Spreng. [25], and the aerial parts of *Selaginella labordei* Hieron. ex Christ. [31]. Isolation of 7-*O*-methyl-derivative of 2,3-dihydroochnaflavone (**8**) from the leaves of *Ochna beddomei* Gamble [32], *Ochna obtusata* DC. [26] has been reported. The stem bark of *Ochna beddomei* Gamble. yielded 7,4',7"-tri-*O*-methyl-2,3-dihydroochnaflavone (**9**) [23]. The isolation of 6,6"-dimethyl-2,3-dihydroochnaflavone (**10**) has been reported from the aerial parts of *Selaginella labordei* Hieron. ex Christ. [31]. 7-*O*-methyl- derivative of tetrahydroochnaflavone (**11**) was derived from the leaves of *Ochna beddomei* Gamble. [32] and *Quintinia acutifolia* Kirk [33]. Reports are also available about 7,7"-di-*O*-methyl-2,3,2",3"-tetrahydroochnaflavone (**12**) and 2,3,2",3"-Tetrahydroochnaflavone (**13**) from the leaves of



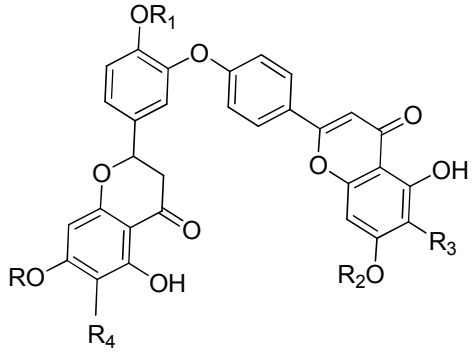
3 R = H R_1 = Me R_2 = H R_3 = H

4 R = H R_1 = H R_2 = Me R_3 = OH

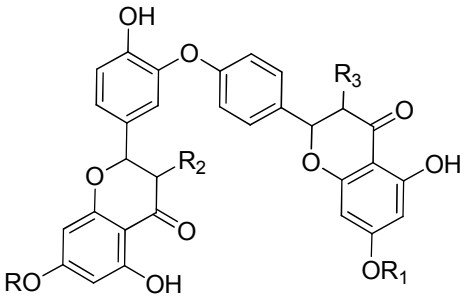


5 R = H

6 R = Me



	R	R_1	R_2	R_3	R_4
7	H	H	H	H	H
8	Me	H	H	H	H
9	Me	Me	Me	H	H
10	H	H	H	Me	Me



	R	R_1	R_2	R_3
11	Me	H	H	H
12	Me	Me	H	H
13	H	H	H	H
14	H	H	OH	OH

Fig. 2. Structures of derivatives of ochnaflavone isolated from different plants

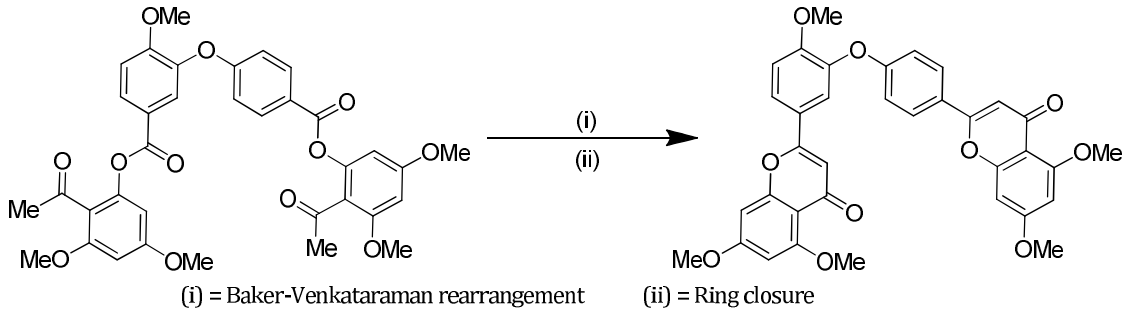
Q. acutifolia, [34]. Flavanol-flavanol derivative, the 5,7,4′,5″,7″-pentahydroxy-biflavanol (14) derivative, was isolated from *Hypnum cupressiforme* Hedw [35,36].

All of the isolated derivatives so far are of –*O*-methyl- nature with an exception of 6,6"-dimethyl-2,3-dihydroochnaflavone (**10**). The prenylated, pyranyl and other substituents (with exception of methoxy or methyl derivatives) of compound **1** have neither been synthesized nor isolated, implying more biological activities to be unveiled (anticipated). From this, it can be concluded that, although compound **1** and derivatives have shown very impressive biological activities (to be discussed in the following sections), less is known concerning the derivatives that are not yet isolated/synthesized, thus, their pharmacological profiles remain masked.

2.2 Synthetic Sources

Since isolation from natural sources is not the only means to obtain phytochemicals, different synthesis methods have been developed as an alternative source. Since these compounds are available in small amounts from their natural sources, the synthesis will ensure QSAR (Quantitative Structure-Activity Relationship) studies, thus, revealing their pharmacological capabilities.

As it is, compound **1** is comprised of -C-O-C- between its two flavone moieties, strategically its synthesis may be carried out through the Baker-Venkataraman rearrangement of the corresponding diphenyl ether dialdehyde, followed by flavone nuclei building [18] as illustrated in Scheme 1.



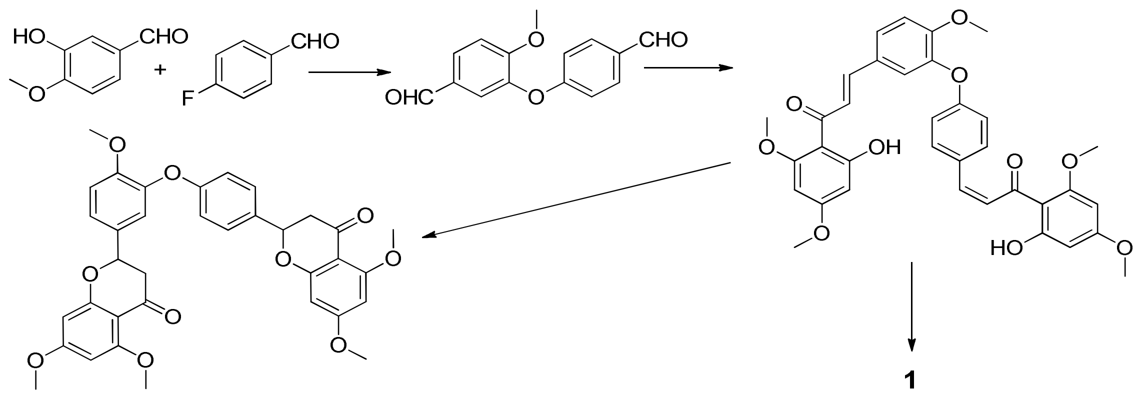
Scheme 1. Synthesis of *penta-O*-methylochnaflavone [18]

Another approach would be building up of the diaryl ether bridge by a simple nucleophilic aromatic substitution reaction, followed by diflavonyl nuclei building as illustrated in Scheme 2 [37].

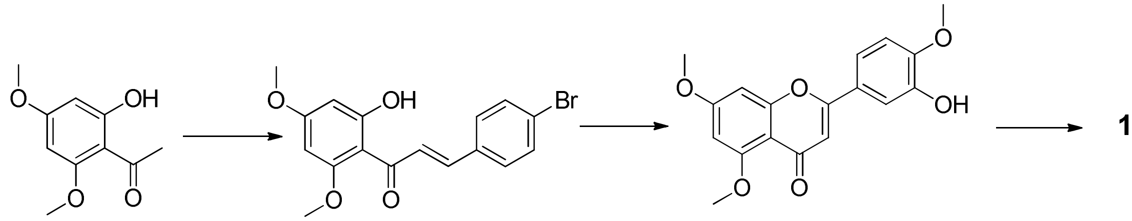
The third approach was first building up of the flavone nuclei then through Ullman coupling diaryl ether linkage was achieved as illustrated in Scheme 3 [38].

Synthesis of derivatives of compound **1** focused on the dihydro and/or methoxy substituents, thus, synthesis of 2,3,2'',3''-tetrahydroochnaflavone and its methoxy derivative was reported by Yingpeng et al. and Ndoile and van-Heerden, respectively [37, 39].

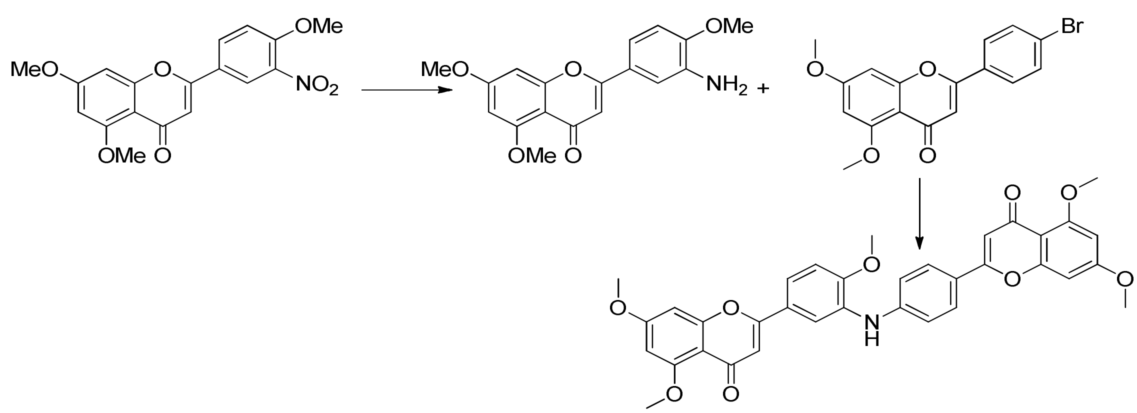
Analogs of compound **1** that are composed of -C-S-C- and -C-NH-C- interflavonoid linkage have been reported by researchers [40]. Stepwise building up of the target biflavone molecules has been illustrated in Schemes 4 and 5 for -C-NH-C- and -C-S-C- analogs, respectively.



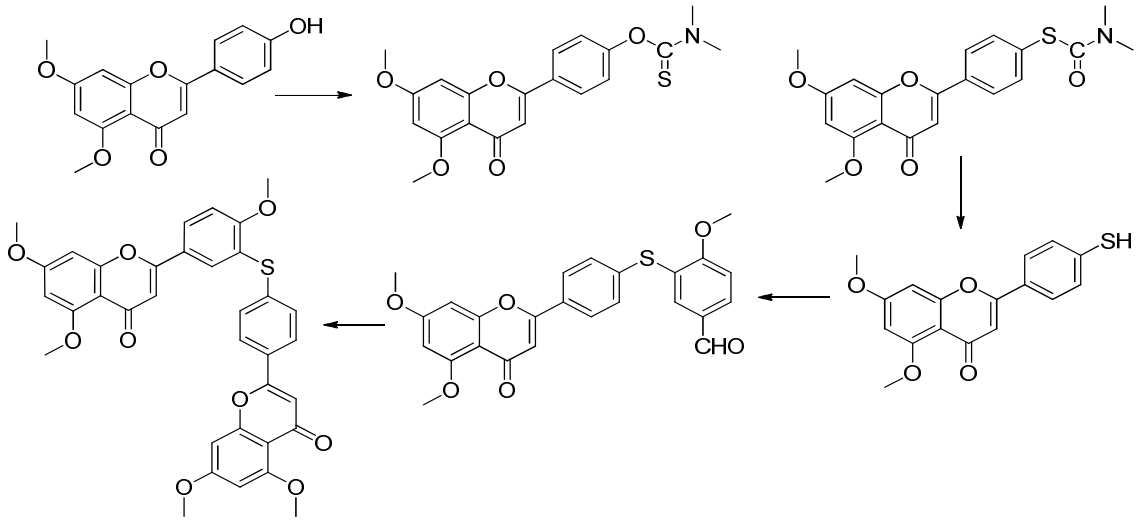
Scheme 2. Synthesis of ochnaflavone and *Penta-O*-methyl-2,3,2'',3''-tetrahydroochnaflavone [37]



Scheme 3. Synthesis of Ochnaflavone [38]



Scheme 4. Synthesis of –C-NH-C- Analog of Ochnaflavone [40]



Scheme 5. Stepwise Synthesis of –C-S-C- Analog of Ochnaflavone [40]

It should be noted that, concerning structural activity relationship studies, substituents like benzoyls, prenyls, pyranyls and others have not been synthesized, thus, their pharmacological effects are not established till date.

3. PHARMACOLOGY

3.1 Molecular Mechanisms of Biflavonoids

Nowadays, cancer has become the most active area of research especially in developing novel strategies to overcome the disease. One crucial point to develop an effective anticancer drug is understanding of signaling mechanisms inside and around these cancer cells. Thus the following sections will show the molecular mechanisms of biflavonoids in overcoming cancer.

Biflavonoids have displayed a spectacular broad spectrum of biological activities; thus, can be regarded for chemopreventive or as therapeutic agents against cancer, therefore, proved to be beneficial to human health [41-43]. Biflavonoids have shown effective control mechanisms against cancer, among all other diseases. Normally the development of cancer is considered to be complex and involves many steps where discrete cellular and molecular changes occur, however, for the sake of simplicity, three major stages are described.

Initiation is the very first and rapid stage that involves exposure and interaction of cells (DNA) to a carcinogenic agent. Promotion is the second stage marked by persistence and replication of abnormal cells; it is a relatively long phase than the initiation. The final phase is progression, marked by the gradual conversion of malignant to neoplastic cells, increased invasiveness, metastasis and formation of new blood vessels [44].

In the efforts to understanding cancer, one major and exciting innovation was the discovery and identification of genes that can transform to a tumour cell under favorable conditions (oncogenes). These include GTP-binding proteins, protein kinases, and nuclear transcription factors [45,46]. The discovery of Protein- Tyrosine Kinases (PTKs) opened up new doors for cancer research and brought a clear understanding of the events taking place in the cancer cells. These enzymes catalyse phosphorylation of tyrosine which induces the cascade of altered cell parameters (characteristic of transformed cells) [47-51].

Biomolecular activities of anticancer agents involve anti-oxidation effects (inactivation of oxygen radicals), electrophils binding, protective enzymes induction, increased rate of apoptosis, inhibition of cell proliferation, lipid peroxidation, angiogenesis and DNA oxidation, and H-donation.

Biflavonoids may exert their protective effects against cancer via interaction with enzymes responsible for the phase one activation. These enzymes (CYP1A1 and CYP1A2) activate a

large number of procarcinogens to reactivate intermediates to interact with nucleophiles and thus trigger the development of cancer. In doing so, they exert a protective activity against cellular damage induction caused by activation of carcinogens. Another mechanism would be induction of metabolizing enzymes such as glutathione S-transferases (GST) by which carcinogens are detoxified and eliminated from the body [52-54].

Biflavonoids may show antiproliferative effects that involve inhibition of pro-oxidants, the process causing tumour promotion. ROS and growth promoting oxidants are major catalysts involved in tumour promotion and progression [53,54], thus biflavonoids have shown to be effective against COX, and therefore inhibit proliferation of tumour cells [38]. Cell cycle arrest accounts for the anticacinogenesis effects of biflavonoids. With mitogenic signals, cells initiate a series of regulated steps that allows transverse of the cell cycle, with Cyclin-Dependent Kinases (CDKs) acting as key regulators in cell cycle progression. Thus, alteration and/or deregulation of CDKs are pathogenic seals of neoplasia. Compounds that inhibit or modulate CDKs are of great interest in cancer researches as potential therapeutic agents [55,56].

Induction of apoptosis is another anticancer activity that is exerted by biflavonoids. This involves elimination of damaged and/or unwanted cells. The process is regulated tightly by a set of genes that promote apoptosis cell survival, mediated by a highly organised network of enzymes and their inhibitors responding to noxious stimuli. Thus, dysregulation of apoptosis is considered a key point in oncogenesis. Biflavonoids have demonstrated this ability by inhibiting the activity of DNA topoisomerase I/II, decrease of ROS and downregulation of nuclear transcription factor kappa B (NF-κB). The following sections will discuss in detail the anticancer and anti-inflammatory activities of ochnaflavone, its derivatives and analogues [57].

The advancement of pharmacology has provided more evidences through laboratory testing on the inhibitory nature of compound **1**. Compound **1** inhibited rat platelet phospholipase A_2 (PLA2), rat peritoneal macrophage arachidonate release and proliferation of lymphocyte [58]. Moreover, the compounds regulated ERK and MMP-9 proteins thus inhibiting the proliferation of smooth muscle cells [59,60]. Furthermore, they blocked phosphatidylethanolamine (PE) degradation in rat liver microsome induced with CCl_4 [61]. Last but not the least; the compounds strongly inhibited degranulation reaction, thus, providing a basis for design and development of novel anti-inflammatory drugs. The mechanism through which these compounds exhibit these inhibitory activities are not limited to affecting NF-κB and ERK pathways, down regulation of inducible nitric oxide, arachidonate 5-lipoxygenase and cyclooxygenase (COX)-2 [59, 60].

So far, a wide range of pharmacological activities of ochnaflavone and derivatives have been demonstrated, thereby attracted an increased attention from researchers of different fields. This paper aims at profiling the sources, derivatives and analysis on recent progress in pharmacological properties of ochnaflavone and its derivatives, thus, points out future perspectives and issues surrounding these compounds, therefore provide a stimulant for further research.

As in ochnaflavone, most pharmacologically relevant natural product molecules are composed of diaryl ether group. This structural feature is the part of various significant pharmaceuticals with antibiotic activities, such as vancomycin, teicoplanin, the antiviral peptide K-13 and the antitumoral bouvardin [62]. Ochnaflavone, its derivatives and analogs have impressive biological activities including anti-inflammatory, anticancer and anti-HIV, as described in the following paragraphs.

3.2 Anti-inflammatory and Anticancer Activity of Ochnaflavone, Derivatives and Analogues

Researches in anti-inflammatory and anti-tumor activities are ongoing, and new ideas are developed due to advancement in technology. Based on early researches, biflavonoids appear to utilize a variety of mechanisms to manifest the observed biological activity. For instance, compound **1** and derivatives have been proved to suppress the activity of proinflammatory enzymes such as cyclooxygenase-2 and lipoxygenase-5 in reducing inflammation. These compounds inhibited cyclooxygenase-2 (COX-2) in BMMC (Bone Marrow-Derived Mast Cells) at IC_{50} = 0.6 μM, additionally, they also inhibited leukotriene C4 production (LTC4) at IC_{50} = 6.5 μM, indicating dual inhibitory activity (cyclooxygenase-2/5-lipoxygenase). Moreover,

the compounds suppressed degranulation reaction at IC_{50} = 3.0 μM [63].

Ochnaflavones have been known to suppress hypertrophy and proliferation in smooth muscle cells. The effect of the compounds on HASMC (human aortic smooth muscle cells) has been investigated by Suh et al. [58]. It is well understood that the occurrence of atheromas on the walls of arteries (atherogenesis) may be caused by infection on vascular smooth muscle cells (VSMC). Therefore, any antiproliferatory molecule will be able to treat the condition. The ability of these compounds to exert anti-artherogenic activity has been correlated to the inhibition of proliferation of VSMC through down regulation of metalloproteinase-9 (MMP-9) [59]. However, this is not the only mechanism through which these compounds show their anti-inflammatory activities. Others include inhibition of LPS-induced iNOS expression. Total inhibition of lipopolysaccharide (LPS)-induced iNOS expression in mouse macrophage cells at IC_{50} = 5.46 M was also observed. These compounds exhibited the reported activity by blocking the inhibition of NF-κB transcription factor binding activities [60].

An irreversible inhibition of lymphocyte proliferation was observed when ochnaflavone was treated on both Con A and LPS induced lymphocytes proliferation. The activity of ochnaflavone has been demonstrated to be superior compared to that of apigenin and quercetin. Thus, the inhibitory effects of ochnaflavone persisted even after the washing of the cells but the proliferation activity was restored after washing in the case of apigenin and quercetin treated cells. The suppression activity of ochnaflavone persisted up to 48 hours after incubation, while that of apigenin was gradually diminishing depending on the time. Quecetin and apigenin could only inhibit both Con A- and LPS induced lymphocyte proliferation while ochnaflavone, on the other hand, inhibited the proliferation of both cells irreversibly [64].

Among various inflammatory mediators secreted upon activation of macrophages is arachidonic acid (AA) which depending on location, species and stimulant is converted to eicosanoids with pro-inflammation properties like PGE2, LTC4 and 5-HETE. Thus, the inhibition of AA secretion from activated macrophages is considered as a key point in anti-inflammatory potential of a given compound. In a study by Lee et al. [57], ochnaflavone inhibited AA release in PMA-induced cells at IC_{30} = 1.5 uM and in A 23187-induced cells at IC_{30} = 8.3 uM. Apigenin which is a monomer of compound **1**, did not show any significant inhibition. Generally, ochnaflavone inhibits AA release by blocking PLA2 pathway [59]. Inhibitory effects of ochnaflavone on COX-2 catalysed production of PGE2 from LPS-activated RAW 2647 cells were observed at IC_{50} value of 1.08 μM [38]. Inhibition of phospholipase A2 (PLA2-IIA) would result into inflammation reduction, thus, ochnaflavone showed to be seven fold stronger than amentoflavone [38].

Another mechanism includes cell cycle arrest and apoptosis induction; evidenced by induction of DNA fragmentation, caspase-3, -8 and -9 activation, and ADP-ribose polymerase cleavage. The growth inhibitory activity of these compounds on cultured human colorectal cancer cells (HCT-15) at IC_{50} = 4.1 μM [65].

The effect of ochnaflavone on the fungal arthritis was determined by examining T cell immunoregulation. The compound inhibited the growth of the mice footpad edema at the peak of a septic state by 45% at 2 mg dose. The observed inhibition was attributed by T helper 1 cytokines suppression (causing Th2 dominance). There is a relationship between arthritis severity and macrophages (which produce NO and thus aggravating joint disease) activation and abundance. The compound exhibited these activities by reducing the population of these macrophages, for instance, at 40 μg/mL of the compound, the population of macrophage was reduced by 70% [66].

The protective effects of ochnaflavone was analysed by pre-treating rat liver microsomes with the compound at 2-16 μM. Upon CCl_4-induced acute liver injury, the marked reduction in the levels of degradation phosphatidylethanolamine (PE) was observed. Furthermore, the compound exhibited inhibition on rat platelet sPLA at IC_{50} = 3.45 μM and lipid peroxidation at IC_{50} = 7.16 μM [61].

2″,3″-dihydroochnaflavone, a flavone-flavanone biflavonoid showed cytotoxicity at IC_{50} = 17.2 μM against murine Ehrlich carcinoma and at IC_{50} = 89.0 μM against human leukemia K562 cells. Furthermore, the acetyl and methyl derivatives of 2″,3″-dihydroochnaflavone exhibited low cytotoxicity against the same cells. Moreover, 2″,3″-dihydroochnaflavone and its acetyl derivative showed to be inhibitors of DNA

topoisomerases I and II – α in the relaxation and decatenation assays. 2″,3″-dihydroochnaflavone in a test with topoisomerase I, showed to be a DNA interacting agent, that is, it may cause unwinding of the DNA, upon spectrophotometric titration, a pronounced hypochromic effect was observed [22].

The imbalance between the inflow, neutralization and the outflow of Reactive Oxygen Species (ROS) may result into oxidative stresses, leading to various pathologies, but, under extreme conditions, cell death may occur. Generally, biflavonoids have shown such robust neuroprotection against ROS- induced stress. Evaluation of the neuroprotective effects of ochnaflavone showed strong neuroprotection activity against cytotoxic insults induced by oxidative stress and amyloid β. These results, however, suggests the therapeutic potential of the compound against ischemic stroke and Alzheimer's disease (neurodegenerative diseases). It was further revealed that the compound has no antioxidative effects, thereby, indicating that the observed neuroprotection might be through blockage of cell death cascades. Inhibiting cell death cascades was effected by blocking staurosporine (known to mediate apoptosis) induced cytotoxic stress (at 10 μM). However, the compound exerted minimal effects at 0.4–10 μM on the etoposide-induced cell death [67].

The -C-NH-C- and -C-S-C- analogs of ochnaflavone have been synthesized, their inhibitory effects on cyclooxygenase-2 (COX-2) mediated prostaglandin E2 production was observed to be weaker than that of ochnaflavone. However, strong inhibitory effects on nitric oxide production on lipopolysaccaride (LPS)-treated RAW 264 7 cells were observed [40]. As far as pharmacological properties of analogs are concerned, a wide range of bioactivities accompanied by their mechanisms need to be investigated.

3.3 Other Biological Activities

Based on the HIV-1 Reverse Transcriptse (RT) and cell assays, 7″-*O*-methylochnaflavone and 7″-*O*-methyl-2″,3″-dihydroochnaflavone were evaluated for their anti HIV activities. The compounds exhibited EC_{50} of 2.0 and 0.9 μg/mL in the syncytium assay, respectively, while in the HIV-1 RT assay, IC_{50} 2.0 and 2.4 μg/mL were observed, respectively. The observed activities for both compounds were higher than that of nevirapine, a non-nucleoside RT standard inhibitor. 2,3,2″,3″-tetrahydroochnaflavone exhibited significant cytotoxicity effects against human nasopharynx carcinoma cells (KB) [68, 69].

Antibacterial activity of ochnaflavone and 7-*O*-methylochnaflavone against *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Staphylococcus aureus* indicated that gram-negatives are more susceptible to chemical attack than gram–positives. Thus, the former showed to be more susceptible to ochnaflavone than the latter with MIC = 31.3 μg/mL (*P. aeruginosa*) and 62.5 μg/mL (*S. aureus*) [70]. Significant antitubercular activity was displayed by triclisinone a 2'-hydroxy-4'-methoxyochnaflavone (**5**) at MIC = 62.5 μg/mL against *Mycobacterium tuberculosis* [29].

Despite the impressive biological activities demonstrated by ochnaflavone, derivatives and analogs, the absorption and distribution properties of these compounds in animals are not yet known. Other biflavonoids investigations include amentoflavone and ginkgetin as analgesics, where potent activity against writhing was observed upon intraperitoneal (i.p.) injection and not through oral administration [71,72]. Similarly, by i.p. injection, antinociceptive activity of I3, II8-binaringenin was observed [73]. Through i.p. injection, anti-inflammatory activity of amentoflavone revealed to be ½ - ⅕ of that of the standard drug indomethacin [72] against acetic acid-induced writhings in mice. Arthritic inflammation in rats was reduced following i.p. injection at 5-20 mg/kg/day of ginkgetin [74].

Generally, unavailability of data on the absorption and distribution of biflavonoids in humans/animals is a challenge to the field of pharmacology. Upon oral administration, morelloflavone and 2,3,2″,3″-tetrahydroamentoflavone exhibited *in vivo* anti-inflammatory activity [75,76]. In other studies, some biflavonoids have shown a reduction of/no activity on oral administration, implying low oral bioavailability of these compounds [77,78]. Intraperitoneal administration has proven to show superior anti-inflammatory activity than topical and oral administration, thus, these studies could be done with ochnaflavone, its derivatives and analogs to establish their effective administration method [71,72,79].

4. PHARMACOKINETICS

Despite the impressive biological activities of the ochnaflavone, its analogs and derivatives, the pharmacokinetic studies have not yet been done; therefore, this review serves as an eye opener to researchers on this very important area of research.

5. CONCLUSION

It can be reasonably depicted from the information cited above that ochnaflavone, its derivatives and analogs possess spectacular broad range of pharmacological activities, including but not restricted to anti-inflammation, anti-tumor, antimicrobial activities, without forgetting effects on the central nervous and cardiovascular systems.

These impressive pharmacological properties displayed by these compounds are expressed through modulation of various enzymes/proteins with key functions in the cells. However, the pharmacokinetics of ochnaflavone and derivatives are not yet revealed. The effects of structural modifications, synthesis of precursors and introduction of pharmaceutical additives to enhance their bioactivity/bioavailability are yet to be studied. Thus, development of lipidic formulations that will enhance their sufficient solubility in aqueous media to allow adequate and reproducible absorption from the Gastro-Intestinal Tract (GIT) following oral administration is recommended. The use of carriers like liposomes, microspheres, nanoparticles, transferosomes, ethosomes are reported for successful modified delivery of insoluble drugs. On the other hand, phytochemicals like quercetin, genistein, naringin, sinomenine, piperine, glycyrrhizin and nitrile glycoside have shown capability to enhance bioavailability, thus to improve bioavailability of ochnaflavone, derivatives and analogues. The use of carriers or incorporation to the above-mentioned phytochemicals is highly recommended.

Although ochnaflavone and derivatives have been isolated and identified from many plants belonging to different families, the recovery and the reproduction of these plants are very costly in cases of demand for large amounts of samples. Therefore, a solution to address this challenge is highly necessary. This might involves synthesis (biological or chemical), allowing formulations preparation, SAR studies, Absorption-Distribution - Metabolism - Excretion, and toxicological studies to be carried out.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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