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Review Paper

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

ABSTRACT

Aims: To profile and address matters surrounding ochnaflavone, 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, anti-tumour, anticancer, anti HIV and antimicrobial.
Conclusion: In this paper, an overview summary of ochnaflavone, derivatives and analogues from past to present is given. 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 of the perceived shortcomings of orthodox medicines [1]. The therapeutic effects of these herbal medicines could be attributed by 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. 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 has 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 [2].

Normally these compounds are not the required or essential nutrients to the body to sustain life but their ability to prevent and fight against diseases has been the focus of researches nowadays. Among phytochemicals that have recently attracted attention of scientists worldwide, include flavonoids. This class of compounds are widely distributed among plants, especially in vegetables, fruits and many others. Normally, these compounds are lower molecular weight polyphenolics shown to possess many health benefits to humans [3-5].

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 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. Others constitute the linkage between the two ring A (AA biflavonoids) or between the two C rings (3,3”-CC biflavonoids). The most rarely observed biflavonoids from nature are the ones with linkage between the two B rings (BB biflavonoids) [6].

The journey towards biflavonoids isolation began when ginkgetin was isolated from the leaves of maidenhair tree (Ginkgo biloba L.,) by Furukawa in 1929, however, to date, the numbers and diversity of biflavonoids keep on increasing. This is due to the structural diversity arising from differences in the position and nature of interflavonoid linkage regardless of the substitution pattern. The diversity among biflavonoids increases further with the presence and type of the substituent/ functional groups and stereogenic centers in their skeleton. So far, about 200 biflavonoids have been isolated from different Gymnosperm and Angiosperm species. Few families such as Anacardiaceae,

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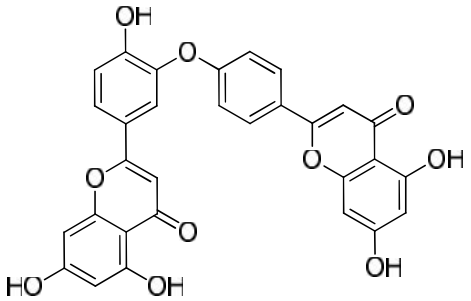
Berberidaceae, Burseraceae, Caprifoliaceae, Casuarinaceae, Euphorbiaceae, Guttiferae (especially Garcinia), Haemcdoraceae, Iridaceae, Labiatae, Leguminosae, Loganiaceae, Ochnaceae, Piperaceae, Rhamnaceae, Rutaceae, Salicaceae, Thymelaeaceae and Velloziaceae have been reported to be sources of biflavonoids. This is a clear indication that the pathway to which biflavonoids are biosynthesized is more specialized and take 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 [7,8].

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 variation. These compounds have shown an array of pharmacological properties including antioxidant, antiproliferative, or anti-inflammatory, indicating their potentiality in pharmacological industry [9-11].

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 and group [12]. The compound and/or derivatives have since then been isolated from many other plant species especially those belonging to the family Ochnaceae, genus Ochna [13].



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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.

The isolation of ochnaflavone (**1**) has been reported from various plant species such as *Lonicera japonica* Thunb. (Leaves) [14], *Cespedesia macrophylla* Seem. (Leaves), *Cespedesia spathulata* (Ruiz & Pav.) Planch. (Leaves) [15], *Luxemburgia nobilis* Eichl. (Aerial parts) [16, 17]. *Ochna beddomei* Gamble. (Leaves and stem bark) [15]. *Ochna integerrima* (Louri) Merr. (leaves) [18], *Ochna lanceolata* Spreng. (Stem bark) [19], *Ochna obtusata* DC (Leaves) [15, 20], *Ouratea staudtii* Van Tiegh. Ex Keay. (Aerial parts) [21].

Derivatives of compound 1, among others, 7-*O*-methylochnaflavone (**3**) is obtained from the leaves of both *Lonicera japonica* Thunb. and *Ochna integerrima* (Louri) Merr. [22]. From the leaves of both *Cespedesia macrophylla* Seem. and *Cespedesia spathulata* (Ruiz & Pav.) Planch., 7”-*O*-methylochnaflavone (**4**) have been isolated [15]. 2′-hydroxy-4′-*O*-methyl derivative, triclisinone (**5**) has been reported from aerial parts of *Triclisia gilletii* [23].

The dihydro derivatives of compound **1**, such as 2”,3”-Dihydroochnaflavone (**6**) have been obtained from leaves of *Luxemburgia nobilis* Eichl. and aerial parts of *Luxemburgia octandra* St. Hil. [17]. The Isolation of the methoxy derivative of dihydroochnaflavones, viz 7”-*O*-methyl-2”,3”-dihydroochnaflavone (**7**), has been reported from the leaves of *Ochna integerrima* (Louri) Merr. [24]. 2,3-dihydroochnaflavone (**8**) was reported from leaves and stem bark of *Ochna beddomei* Gamble.

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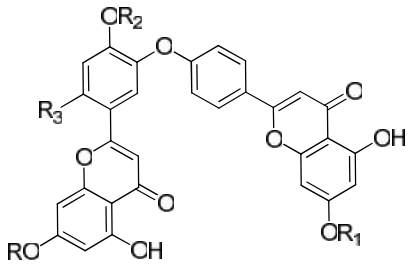
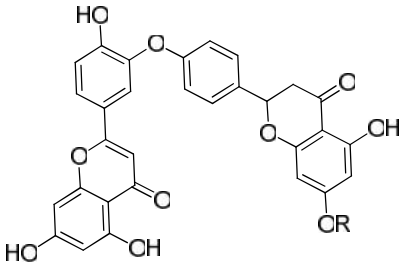
[15, 25], stem bark of *Ochna lanceolata* Spreng. [19], leaves of *Ochna obtusata* DC. [20], *Selaginella labordei* Hieron. ex Christ. (Aerial parts) [26], *Luxemburgia nobilis* (Leaves) [16]. Isolation of 7-*O*-methyl- derivative of 2,3-dihydroochnaflavone (**9**) from leaves of *Ochna beddomei* Gamble. [15], *Ochna obtusata* DC. [20] has been reported. The stem bark of *Ochna beddomei* Gamble. yielded 7,4',7”-tri-*O*-methyl-2,3-dihydroochnaflavone (**10**) [15, 25]. From aerial parts of *Selaginella labordei* Hieron. ex Christ. the isolation of 6,6”-dimethyl-2,3-dihydroochnaflavone (**11**) has been reported [26]. 7-*O*-methyl- derivative of tetrahydroochnaflavone (**12**) was isolated from leaves of both *Ochna beddomei* Gamble. and *Quintinia acutifolia* Kirk [15]. From the leaves of the latter, 7,7”-di-*O*-methyl-2,3,2”,3”-tetrahydroochnaflavone (**13**) and 2,3,2”,3”-Tetrahydroochnaflavone (**14**) have been reported [27]. Flavanol-flavanol derivative, the 5,7,4′,5″,7″-pentahydroxy-biflavanonol derivative, was reported from *Hypnum cupressiforme* Hedw. [28, 29].





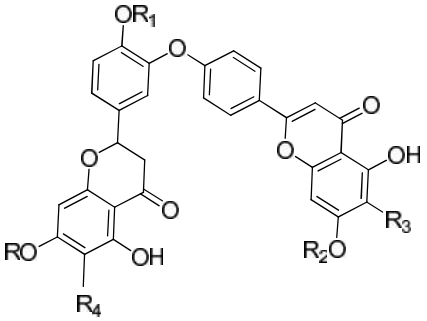
3 **R** = Me **R₁** = H R_2 = H R_3 = H

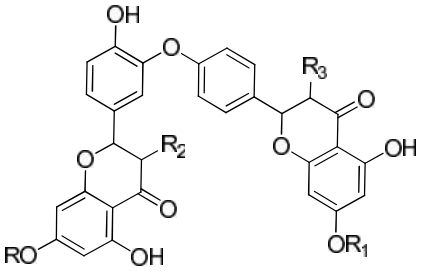
4 **R** = H **R₁** = Me R_2 = H R_3 = H

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

6 **R** = H

7 **R** = Me





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

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

All of the isolated derivatives so far are of –*O*-methyl- nature with an exception of 6,6”-dimethyl-2,3-dihydroochnaflavone (**11**). 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 synthetic methods have been developed as an alternative source. Since these compounds are available in small amounts from their natural sources, synthesis will ensure QSAR (Quantitative Structure-Activity Relationship) studies, thus, revealing their pharmacological capabilities.

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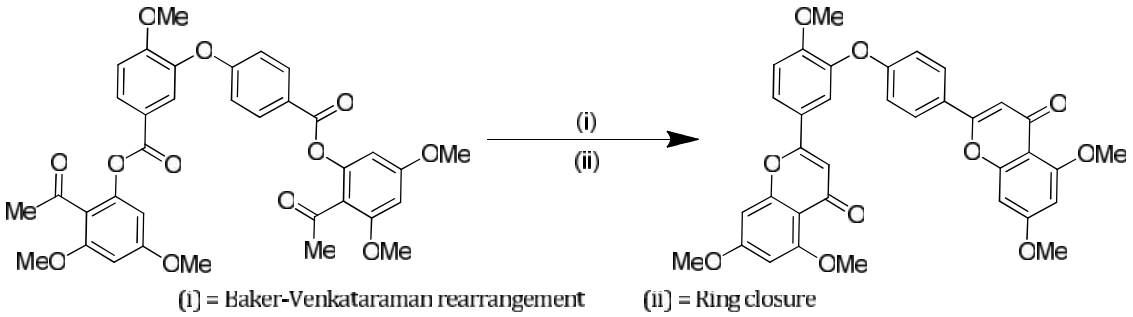
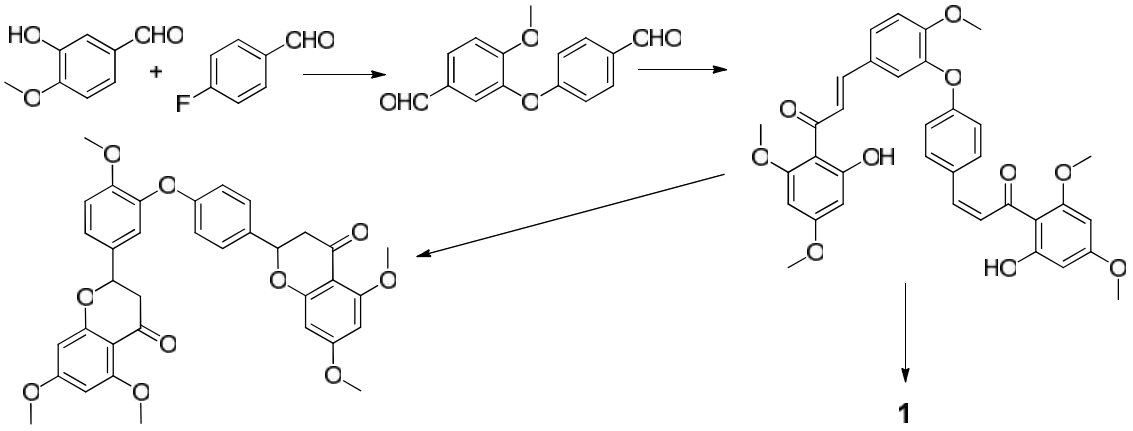
As it is, compound **1** is comprised of -C-O-C- between its two flavone moieties, strategically its synthesis may be carried out through Baker-Venkataraman rearrangement of the corresponding diphenyl ether dialdehyde, followed by flavone nuclei building [12] as illustrated in **Scheme 1**.



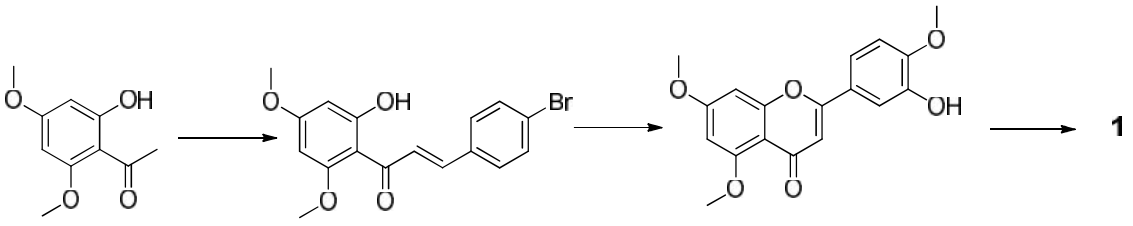
Scheme 1: Synthesis of *penta-O*-methylochnaflavone [12]

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** [30].



Scheme 2: Synthesis of ochnaflavone and *Penta-O*-methyl-2,3,2”,3”-tetrahydroochnaflavone [30]

Yet another approach was first building up of the flavone nuclei then through Ullman coupling diaryl ether linkage was achieved as illustrated in **Scheme 3** [31].



Scheme 3: Synthesis of Ochnaflavone [31]

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 (2015), and Ndoile and van-Heerden 2013 respectively [30, 32].

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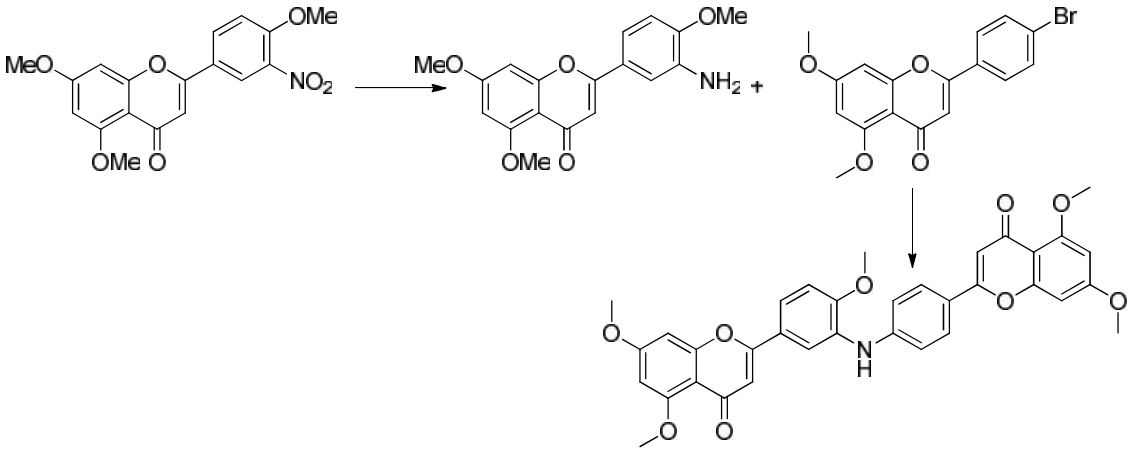
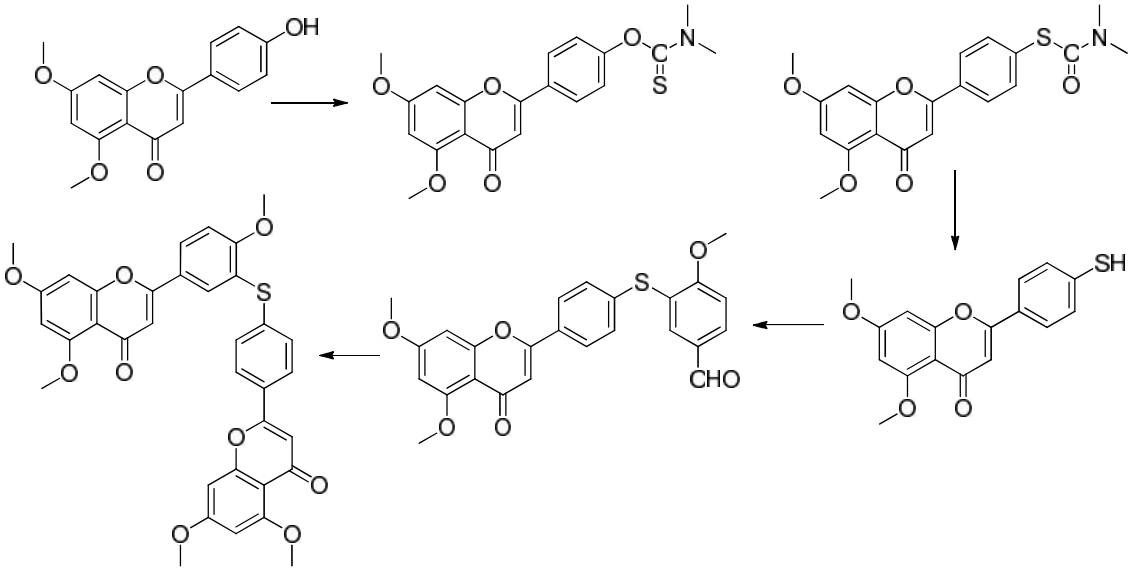
Analogs of compound **1** that are composed of -C-S-C- and -C-NH-C- interflavonoid linkage have been reported [33]. Stepwise building up of the target biflavone molecules is as illustrated in **schemes 4** and **5** for -C-NH-C- and -C-S-C- analogs respectively.



Scheme 4: Synthesis of –C-NH-C- Analog of Ochnaflavone [33]



Scheme 5: Stepwise Synthesis of –C-S-C- Analog of Ochnaflavone [33]

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 not known/established.

3. PHARMACOLOGY

The advancement of pharmacology has provided more evidence through laboratory testing on the inhibitory nature of compound **1**. Compound **1** inhibits rat platelet phospholipase A_2 (PLA2), rat peritoneal macrophage arachidonate release and proliferation of lymphocyte [34]. Moreover, the compounds regulated ERK and MMP-9 proteins thus inhibiting proliferation of smooth muscle cells [35, 36]. Furthermore, they blocked phosphatidylethanolamine (PE) degradation in rat liver microsome induced with CCl_4 [37]. Lastly but not the least, the compounds strongly inhibited degranulation reaction, thus, providing a basis for novel anti-inflammatory drugs design and development. The mechanism through which these compounds exhibit these inhibitory activities are but not limited to

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affecting NF-κB and ERK pathways, down regulation of inducible nitric oxide, arachidonate 5-lipoxygenase and cyclooxygenase (COX)-2 [35, 36].

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

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

3.1 Anti-inflammatory, anti-tumour and anticancer activity

Researches in anti-inflammatory and anti-tumor activities are ongoing, new ideas are developed due to advancement in technology. Based on early researches, biflavonoids appear to utilize variety of mechanisms to manifest the observed biological activity. For instance, compound **1** and derivatives have expressed their ability 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 [38].

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 2006. 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) [35]. 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 observed. These compounds exhibited the reported activity by blocking the inhibition of NF-κB transcription factor binding activities [36]. Another mechanism includes cell cycle arrest and apoptosis induction; evidenced by DNA fragmentation induction, 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) was inhibited at IC_{50} = 4.1 μM [39].

An irreversible inhibition of lymphocyte proliferation was observed when ochnaflavone was treated on lymphocytes proliferation induced by both Con A and LPS. 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 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 time dependently. Quecetin and apigenin could only inhibit both Con A- and LPS induced lymphocyte proliferation while ochnaflavone on the other hand inhibited the proliferation on both cells irreversibly [40].

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 (1997), 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, it was discovered that ochnaflavone

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inhibits AA release by blocking PLA2 pathway [35]. Inhibitory effects of ochnaflavone on COX-2 catalysed production of PGE2 from LPS-activated RAW 264.7 cells was observed at IC_{50} value of 1.08 μM [31]. Inhibition of phospholipase A2 (PLA2-IIA) would result into inflammation reduction, thus, ochnaflavone showed to be stronger seven fold than amentoflavone [31].

Ochnaflavone derivatives exhibited potent growth inhibition on leukemia cells, 2,3,2",3"-tetrahydroochnaflavone, 2",3"-dihydroochanaflavone and 7,7”-Di-O-methyl-2,3,2”,3”-tetrahydroochnaflavone actively inhibited the growth of lymphocytic leukemia cells (P388) with IC_{50} = 8.2, 3.1, 8.7 μg/mL, respectively [27, 41].

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 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% [42].

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 [37].

2″,3″-dihydroochnaflavone, a flavone-flavanone biflavonoid showed cytotoxicity at IC_{50} = 17.2 μM against murine Ehrlich carcinoma and 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 [17].

The imbalance between the inflow, neutralization and the outflow of Reactive Oxygen Species (ROS) may result into oxidative stresses, leading into 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 shown that the compound have 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 showed minimal effects at 0.4–10 μM on the etoposide-induced cell death [14].

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 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 was observed [33]. As far as pharmacological properties of analogs is concerned, a wide range of bioactivities accompanied by their mechanisms need to be established/investigated.

3.2 Other biological activities

Basing on HIV-1 RT and cell assays, 7″-*O*-methylochnaflavone and 7″-*O*-methyl-2″,3″-dihydroochnaflavone were evaluated for their anti HIV activity. The compounds exhibited EC_{50} of 2.0 and 0.9 mg/mL in the syncytium assay respectively, while in the HIV-1 RT assay, IC_{50} 2.0 and 2.4 mg/mL were observed, respectively. The observed activities for both compounds were higher than that of vitexin, a non-nucleoside RT standard inhibitor. 2,3, 2″,3″-tetrahydroochnaflavone exhibited significant cytotoxicity effects against human nasopharynx carcinoma cells (KB) [22, 43].

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Antibacterial activity of ochnaflavone and 7-*O*-methylochnaflavone against Escherichia coli, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Staphylococcus aureus* indicated gram-negative are more susceptible to chemical attack than gram–positive. 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*) [44]. Significant antitubercular activity was displayed by triclisinone a 2'-hydroxy-4'-methoxyochnaflavone (**5**) at MIC = 62.5 µg/mL against *Mycobacterium tuberculosis* [23].

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 investigated include amentoflavone and ginkgetin as analgesics, where potent activity was observed upon intraperitoneal (i.p.) injection and not through oral administration [45]. Similarly, by i.p. injection, antinociceptive activity of 3-8″ binaringenin was observed [46]. Through i.p. injection, anti-inflammatory activity of amentoflavone by revealed to be ½ - ⅕ of that of the standard drug indomethacin [9]. Arthritic inflammation in rats was reduced following i.p. injection at 5-20 mg/kg/day of ginkgetin [47]. Generally, there has been a reduction of/no activity on oral administration of these compounds, implying low oral bioavailability of these compounds, thus, same studies could be done with ochnaflavone, its derivatives and analogs.

4. PHARMACOKINETICS

Despite of 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 concluded from the information cited above that ochnaflavone, its derivatives and analogs possess a variety of pharmacological activities, including but not restricted to anti-inflammation, anti-tumor, antimicrobial activities, and also have 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.

5.1 New Research Avenues

Pharmacokinetics of ochnaflavone and derivatives are not yet known, the effects of structural modifications, synthesis of precursors and introduction of pharmaceutical additives to enhance their bioactivity/bioavailability are 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 is very costly in cases of demand for large amounts of samples, therefore, a solution to address this challenge is highly needed. Some of the solutions might be through biological synthesis (which has not been done) and chemical synthesis, allowing formulations preparation, structural modification, Absorption-Distribution-Metabolism-Excretion (ADME), and toxicological studies to be carried out.

Concerning derivatives of ochnaflavone that have not yet been isolated and identified from nature, synthesis could be employed so as to better study these compounds. Moreover, incorporation of nanomaterials to improve their surface and enhance solubility is suggested to create new pharmacological applications.

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