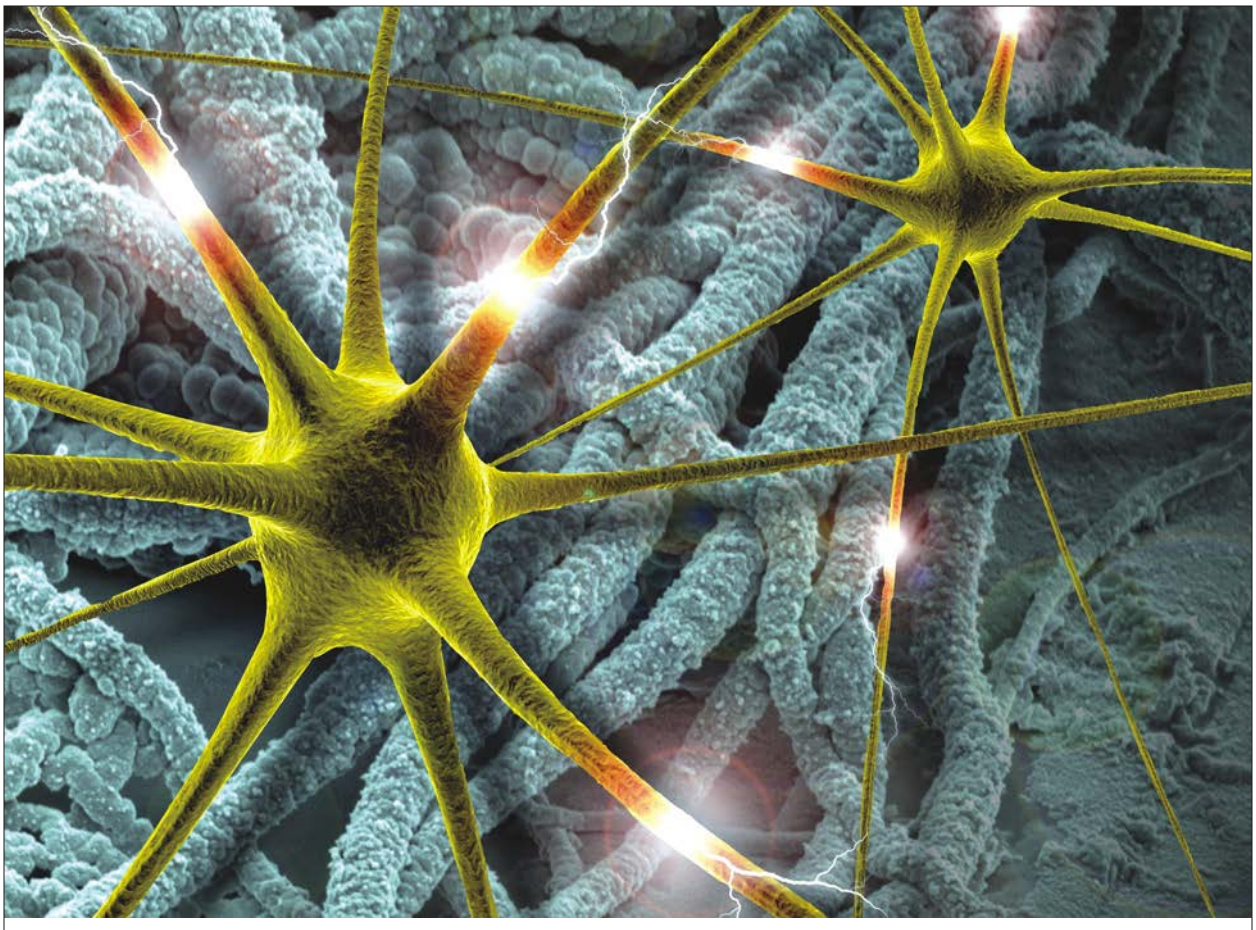

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Review article
Review on Chemistry and Pharmacological Potential of Amentoflavone

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Abstract
Amentoflavone is a polyphenolic compound present in various plants including *Ginkgo biloba*, *Chamaecyparis obtusa* (hinoki), *Hypericum perforatum* (St. John's Wort) and *Xerophyta plicata*. It mainly shows its antagonist activity at κ-opioid receptor and at the allosteric benzodiazepine site of the GABA (A) receptor as a negative allosteric modulator. Its boiling point is 910.00-911.00°C at 760.00 mmHg (est) and melting point is more than 572°F. It occurs in solid state and weight is 538.46. Its chemical formula is $C_3OH_{18}O_{10}$ and molar mass is 538.45 g moL^{-1}. Amentoflavone is analytically observed by various spectroscopical parameters i.e., HPLC, TLC, paper chromatography. Structural determination can be done by UV, NMR and IR parameters. Amentoflavone shows various molecular mechanisms i.e., phosphodiesterase inhibition, muscular strength, acetylcholinesterase inhibition, inhibition of PTP1B, weak vasodilation and also inhibit fatty acid synthesis.

Key words: Amentoflavone, GABA receptors, HPLC, TLC, benzodiazepine sites, UV, NMR, ACH inhibition

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Amentoflavone (bisapigenin coupled at 8 and 3'positions or 3, 8 biapigenin) is a type of bioflavonoid and an important constituent of different plants like *Ginkgo biloba*, *Chamaecyparis obtusa* (hinoki), *Hypericum perforatum* (St. John's Wort)[1] and *Xerophyta plicata*[2,3]. It has been proved for various *in vitro* activities, including antimalarial activity[4], anticancer activity (which may, at least in part, be mediated by its inhibition of fatty acid synthase)[5-7] and antagonist activity at the kopioid receptor (Ke = 490 nM)[8] as well as activity at the allosteric benzodiazepine site of the GABA (A) receptor as a negative allosteric modulator[9]. The molecular activity involves, being a potent inhibitor of CYP3A4 and CYP2C9, which are enzymes responsible for the metabolism of some drugs in the body[3]. It is also an inhibitor of human cathepsin B(1) and ability to modulate the benzodiazepine GABA (A) receptor site[10]. This modality is a potential way to enhance learning and memory since it would disinhibit excitatory neurotransmission. It could also increase the production of testosterone by inducing the release of GnRH at the hypothalamus. Amentoflavone remains in controversy because of its structure and its capacity to cross the bloodbrain barrier[10]. Followed by an evidence as, as GABA (A) antagonism brings along with it a host of potentially dreadful side effects from anxiety to the potential for neurotoxicity. Its peripheral properties are significant enough to warrant further investigation.The *in vivo* pane titration of amentoflavone in to porcine brain, endothelial cell was proved by passive diffusion and its transportation across the porcine brain capillary endothelial cells monolayers (BCEC)[10]. Amentoflavone has also shown its affinity to inhibit binding at serotonin (5HT1Da Ki = 4094 M, 5HT2C Ki = 2555 M), D3dopamine (Ki = 1241 M), dopioid (Ki = 36.5 M) except binding to the GABA receptor site[11] and still there is a need to determine its functional activity through GABA receptor assays.

Basic properties:

- Assay: 95.00-100.00%
- Food chemicals codex listed: No
- Boiling point: 910.00-911.00°C at 760.00 mmHg (est)
- Flash point: 587.00°F. TCC (308.50°C) (est)
- logP (o/w): 3.492 (est)

Synonyms: $C_3OH_{18}O_{10}$, "8-[5-(5, 7-dihydroxy-4-oxochromen-2-yl)-2-hydroxyphenyl]-5,7-",dihydroxy-2-(4-hydroxyphenyl)chromen-4- one flavones.

Physical properties:

- It occurs in the divided solid state
- Melting range of amentoflavone is more than 572°F
- Molecular weight is 538.46
- It is partially miscible in water. Its solubility is measured by gram per liter
- It is pale yellow powder, does not mix well with water

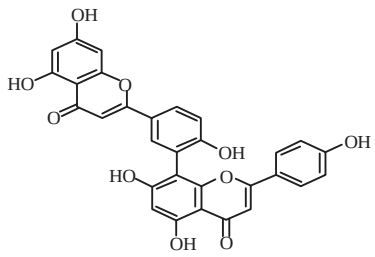
Chemistry and structure:

- Chemical formula: $C_3OH_{18}O_{10}$ (Fig. 1)
- Molar mass: 538.45 g mol^{-1}

Chromatographic analysis: Analysis of amentoflavone was done by many ways these are: TLC, HPLC, HPTLC, paper chromatography, etc.

TLC: The TLC analyses of amentoflavone samples in flavanoid rich fractions were described by Sannomiya *et al.*[12] using silica gel TLC plates on glass (20×20 cm, Aldrich) developed with a solvent mixture composed of $CHCl_3$-CH_3OH (85:15 (v/v)). The spots on the TLC plates were observed under a UV lamp (254 nm). Fractions of similar Retention Factors (RF) were combined, weighed and further analysed using a varian, ProStar HPLC system[13].

HPLC: The HPLC analyses of amentoflavone samples in flavanoid rich fractions were described by Laghari *et al.*[14] using HPLC-ESI-MS/MS. The liquid chromatograph system was equipped with the photo diode array detector (PDA) and a vacuum de-gasser. Separations were made by using hypersil gold C18 (250×4.6 mm, 5 µm) (Thermo electron corpo-ration USA) column and analytical data were evaluated by using the x-caliber data processing system (2.0 SR2). The mobile phase was composed of methanol-acetoni-trile (7:3) (A) and 0.1% v/v formic acid in water (B). The flow rate was



Amentoflavone

Fig. 1: Structure of amentoflavone

1 mL min^{-1}. The gradient programming was as follows; starting from concentration of A at 5% for 5 min and then gradual increase from 5-30% in 10 min. Isocratic step of 5 min and then brought back to 5% in 5 min followed by 5 min for column equilibration. The eluent was monitored using the PDA detector set at three different wavelengths 270, 320 and 360 nm[15].

Isolation and identification of amentoflavone: The isolation and identification of amentoflavone were performed in methanolic. Extract of plant *Selaginella tamariscina*, followed by partitioned sequentially with dichloromethane, ethyl acetate and n-butanol. The active fraction (EtOAc fraction) (3.0 g) was placed on a silica gel (300 g, 4.845 cm) column and eluted using a $CHCl_3$-MeOH-H_2O (12:1:0.1→→8:1:0.1→5:1:0.1→ 2:1:0.1→1:1:0.1→MeOH only) gradient system. The yielded amount of amentoflavon through column chromatography was 83.23 mg and identified by UV, IR, 1H and 13C-NMR data, for amentoflavone, were identical to those reported in literature[14-16].

Structural determination of amentoflavone: The characterization of structural properties of amentoflavone (AF) was achieved by different spectroscopic techniques[14]:

- **UV:** (MeOH) 1max (log e) 332 (9.5)
- **ESIMS (Positive ion):** m/z 539 [M+H]+; m/z 512 [M+Na-18]+, 455 [M+Na-18]+. 1H
- **NMR data:** (CD3OD, 600 MHz) d 6.18 (1H, br s, H-6), 6.38 (1H, s, H-6″), 6.40 (1H, br s, H-8), 6.59 (1H, s, H-3″), 6.60 (1H, s, H-3), 6.72 (2H, d, J = 8.0 Hz, H-5‴, H-3‴), 7.12 (1H, dd, J = 8.0, 1.5 Hz, H-6′), 7.54 (2H, d, J = 8.0 Hz, H-2‴, H-6‴), 7.89 (1H, d, J = 8.0 Hz, H-5′), 7.95 (1H, d, J = 1.5 Hz, H-2′)
- **^{13}C NMR data:** (CD3OD, 600 MHz) d 166.0 (C-2), 102.3 (C-3), 184.6 (C-4), 163.4 (C-5), 98.4 (C-6), 166.4 (C-7), 93.5 (C-8), 159.8 (C-9), 105. 6 (C-10), 123.5 (C-1′), 131.0 (C-2′), 122.0 (C-3′), 161.6 (C-4′), 127.9 (C-5′), 116.6 (C-6′), 166.6 (C-2″), 101.8 (C-3″), 185.0 (C-4″), 163.8 (C-5″), 98.6 (C-6″), 162.8 (C-7″), 106.0 (C-8″), 159.6 (C-9″), 105. 3 (C-10″), 123.5 (C-1‴), 128. 2(C-2‴, C-6‴), 115.4 (C-3‴, C-5‴), 163.0 (C-4‴)

Molecular mechanism: Molecular mechanism of amentoflavone is shown in Fig. 2.

Phosphodiesterase inhibition: Phosphodiesterase (PDE) is an intracellular enzyme which degrades the second messengers cAMP or cGMP. In human adipose tissue, β-2 agonism results as an increase in cAMP which activates lipases that cause a cellular lipid breakdown ("Lipolysis"). By inhibiting the particular phosphodiesterase isoenzyme (PDE3) found in adipose tissue, a compound could theoretically synergize with the adrenergic signalling cascade and induce significant fat loss. Indeed, amentoflavone has demonstrated this capacity in a 1998 Italian study examining the effect of Ginkgo biloba on rat adipose tissue[14].

This study compares the inhibition of cAMP-phosphodiesterase in rat adipose tissue by a mixture of *Ginkgo biloba* biflavones with the effect of individual dimeric flavonoids has been reported in order as amentoflavone>bilobetin>sequoiaflavone>ginkgetin = isoginkgetin[14].

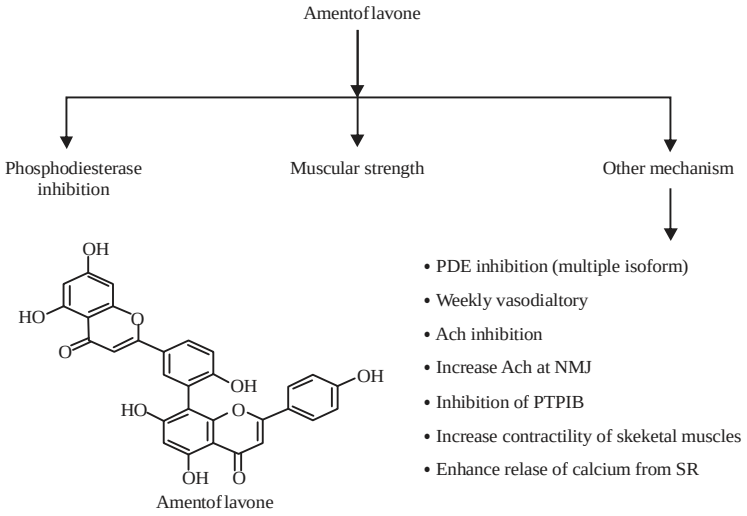


Fig. 2: Molecular mechanism of amentoflavone

A 2006 Planta Medica article also identified amentoflavone as a weak inhibitor of PDE5, although having much greater inhibitory capacity for other isoforms[15,16]. The former PDE is responsible for the metabolism of cGMP, whereas the latter isoforms deal mainly with cAMP. Inhibiting cGMP disposal allows for vascular dilation (i.e., viagra) via smooth muscle relaxation. Inhibiting cAMP metabolism potentiates various transduction cascades, including lipolysis in adipose tissue and enhancing cardiac contractility and speed[15].

Muscular strength: Amentoflavone was recently demonstrated to possess acetylcholinestase inhibiting properties in a 2011 study[16]. By inhibiting AchE, more acetylcholine ligand would be available at the neuromuscular junction, disinhibiting Ach metabolism from being a rate limiting step for muscular contraction. Unfortunately, AchE inhibition alone has not demonstrated an ability to enhance muscular strength in healthy individuals[17]. Fortunately, however, amentoflavone possesses another modality that may synergize with AchE inhibition: enhancing the calcium release from the sarcoplasmic reticulum.

The Ca^{2+} releasing activity of amentoflavone was approximately 20 times more potent than that of caffeine. These results suggest that amentoflavone, which does not contain a nitrogen atom, probably binds to the caffeine-binding site in Ca^{2+} channels and thus potentates Ca^{2+} induced Ca^{2+} release from the heavy fraction of fragmented sarcoplasmic reticulum.

This is a novel mechanism for enhancing muscular contraction and one of the ways in which caffeine increases strength, albeit weakly[18]. Since, amentoflavone is approximately 20 time more potent then caffeine, it is also possible that it could exert greater efficacy in this area.

Other mechanisms: Amentoflavone, in addition to its exceptionally weak ability to inhibit fatty acid synthase[19] and ability to potentiate cAMP in adipose tissue, also possesses another novel metabolic mechanism: Protein tyrosine phosphatase 1B (PTP1B) inhibition[20]. The PTP1B is an negative regulator of the growth promoting cascade induced by tyrosine kinase receptors. By inhibiting PTP1B, amentoflavone dysregulates the downstream pathways activated by various ligands, including those induced by insulin. This could have an exceptionally beneficial effect in relation to insulin insensitivity or just as a means to potentiate insulin itself. Unfortunately, it could also have pro-oncogenic outcomes in those with cancer. Needless to say, any growth promoting compound (estrogen, GH, IGF-1, DHT, etc.) has the capacity to stimulate oncogenesis and so this mechanism should not be hysteria-provoking-especially in light of amentoflavones other anti-cancer modalities (anti-mutagenesis, anti-angiogenesis).

Other mechanisms also involves:

- PDE inhibitions (multiple isoforms)
- Weakly vasodilatory
- Capacity to be potential adrenergic signalling in adipose tissue-enhanced lipolysis
- Acetylcholinesterase inhibition
- Increased availability of acetylcholine at the NMJ
- Enhancing the release of Ca^{2+} from the sarcoplasmic reticulum
- Increased contractility of skeletal muscle
- Inhibition of PTP1B
- Potentiation of insulin signaling and other growths promoting cascades (unknown tissue specificity)

Pharmacological activities

Amentoflavone possesses antiangiogenic activity: The antiangiogenic property of AF has been correlated with biochemical and functional relationship between Vascular Endothilials Growth Factors (VEGFs) and related receptors[21]. The urge of a new generation of agents able to target contemporarily more than one member of the VEGFs might amplify the antiangiogenic response. This research provides a key to overcome most of the difficulties associated with current angiogenesis inhibitors, with better safety profile[22,23].

Radioprotective effect of amentoflavone: The protective effects of amentoflavone against radiation in cells were investigated and examined for cell viability, apoptosis, cell cycling concentrations of intracellular Reactive Oxygen Species (ROS) and relative mitochondrial mass by flow cytometry after 60 Co irradiation. The study was designed with prior treatment of amentoflavone (24 h) to 8 Gy 60 Co γ-ray irradiation significantly inhibited apoptosis, promoted the G2 phase, decreased the concentration of ROS and mitochondrial mass. The result was proved to be as radio protective[24].

Anti-oxidant activity of amentoflavone: The *in vitro* antioxidant activity of amentoflavone was found to be efficacious at •OH-induced oxidative damage DNA (including base and deoxyribose moieties), via deoxy nucleotide radical repairing and Reactive Oxygen Species (ROS). The activity showed effective due to scavenging and repairing approaches, which may ultimately arise from to the stability of

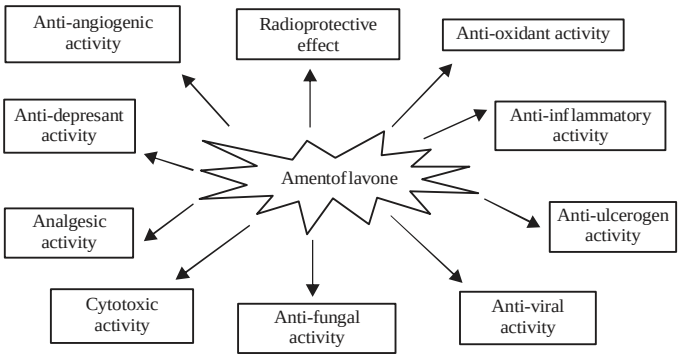


Fig. 3: Pharmacological activity of amentoflavone

Table 1: Different biological sources and uses of amentoflavone

Biological sources	Uses	References
Semecarpus anacardium	Leprosy and ulcers	Jung *et al.*[35] and Lin *et al.*[39]
Chamaecyparis obtusa	Analgesic and anti-RA	Kuo *et al.*[40]
Selaginella tamariseina	Anti-cancer, anti-inflammatory and analgesia	Volz[41] and Pilepic *et al.*[42]
Hypericum perforatum	Burns, skin injuries and neuralgia	Pilepic *et al.*[42]
Hypericum adenotrichum	Cytotoxic and anti-depresant	Horakova and Stolc[43]
Byrsonima crassa	Anti-oxidant and anti-ulcer	Songca *et al.*[44]
Rhus leptodictya	Anti-bacterial	Bais *et al.*[45]
Juniperus communis	Antioxidant and neuroprotective	Bais *et al.*[46] and Bais and Prashar[47]

its oxidized product semi-quinone form. The study proved its protection against DNA damage may be generally responsible for the antioxidant and anti-inflammatory effects[25-27] (Fig. 3).

Other activities: Amentoflavone is also proved for various other pharmacological effects like anti-inflammatory[28-30], anti-ulcerogenic[30], anti-depressant[29], anti-oxidant[30], analgesic[31] and it has cytotoxic activity[32,33]. Besides such biological activity, there have also been reports regarding its biological effects toward microorganisms. Studies have shown that amentoflavone has antiviral activity against influenza, herpes and respiratory syncytial virus (RSV)[34,35] and antifungal activity with the main focus being on phytopathogens, which coincides with the inhibition of phytopathogen infections[36,37]. Amentoflavone possessed antimicrobial activity which are highly effective at human pathogenic fungi but the effect induced by this compound in intracellular condition of *C. albicans*[38] (Table 1).

CONCLUSION

The above study reveals that the amentoflavone is safer at its boiling range of 910.00-911.00°C at 760.00 mmHg (est) and melting range of 572°F. The compound was found to be potent antimicrobial, antifungal, anti-viral, anti-depressant, anti-inflammatory, anti-oxidant, anti-ulcerogenic, analgesic, anti angiogenic, radioprotective and cytotoxic activity. However, further studies are required to prove its safety, efficacy and reliability.

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