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Acetovanillone (Apocynin): A Multifaceted Phenolic Compound with Therapeutic and Biotechnological Relevance

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ABSTRACT

The identification of acetovanillone (apocynin) in hydroethanolic extracts of *Boophone disticha* bulbs represents a notable advance in phytochemical profiling within South African Amaryllidaceae, a family traditionally recognized for its alkaloid diversity. This discovery is further expanded by evidence for acetovanillone in *Crinum graminicola* and *C. buphanoides*, alongside the exclusive detection of piceol in the latter, collectively extending the chemical landscape to aromatic, non-alkaloid phenolics. These compounds, characterized by mild vanilla-like aromas, highlight previously underappreciated facets of Amaryllidaceae's metabolic biodiversity. Importantly, acetovanillone's pronounced anti-inflammatory activity mediated *via* NADPH oxidase inhibition closely aligns with the documented ethno- pharmacological uses of these species for inflammatory conditions. Such phenolic antioxidants expand the pharmacognostic significance of Amaryllidaceae, supporting their therapeutic versatility beyond their alkaloidal content. Advancements in microbial biotransformation have shown *Streptomyces tunisiensis* can sustainably convert ferulic acid to acetovanillone, establishing new biosynthetic routes for industrial production and metabolic engineering targeting nutraceuticals and cosmeceuticals. Beyond plant systems, acetovanillone has shown robust neuroprotective potential, notably binding and inhibiting TDP-43, a protein linked to the pathogenesis of amyotrophic lateral sclerosis, with superior pharmacokinetic profile compared to clinically approved agents. In cardiovascular research, acetovanillone, alone and in combination with carvedilol, has demonstrated potent antioxidative and anti-inflammatory effects, modulating redox-sensitive signalling cascades and reducing myocardial injury. It suppresses NADPH oxidase expression and upregulates Nrf2, HO-1 and SIRT1, key protectors against oxidative cardiac damage, while attenuating inflammatory cytokines and necroptosis. Acetovanillone and its derivatives with antibacterial and antileishmanial activities have a wide range of applications like plant immunity, reproductive biology, hepatoprotection and chemoprevention, underscoring its utility as a chemotaxonomic marker and pharmacological lead for oxidative stress related disorders. Through extensive biochemical and molecular evidence, acetovanillone emerges as a multifaceted bioactive with promise for phytomedicine, drug development and quality control innovation in herbal formulations.

Key words: Acetovanillone, Anti-inflammatory, Antimicrobial, Antioxidant, Apocynin.

Acetovanillone (4-hydroxy-3-methoxyacetophenone) is a small molecule classified under phenolic compounds, widely noted for its inhibitory effect on NADPH oxidase (NOX) and its role in modulating redox biology. The compound's dual role serving both as a therapeutic antioxidant and a modulator of oxidative stress pathways makes it uniquely positioned in the field of redox pharmacology. Traditionally used in African ethno-medicine, its presence in various medicinal pla nts signifies its pharmacological relevance. Its increasing prominence in biomedicine is due to its ability to penetrate biological barriers, modulate key signalling pathways and exhibit minimal toxicity under controlled dosing. This review summarizes key findings on its phytochemical sources, mechanisms of action, therapeutic potentials and emerging applications in drug design, agriculture and biotechnology.

Phytochemical sources and isolation

The identification of acetovanillone (apocynin) in hydroethanolic extracts of *Boophone disticha* (Fig 1) bulbs (Tagwireyi and Majinda, 2017), marks a significant advancement in the phytochemical profiling of South African Amaryllidaceae family. Subsequent screening by Masi *et al.* (2018) revealed, for the first time, the presence of acetovanillone in both *Crinum graminicola* (Fig 2) and *C. buphanoides* (Fig 3), alongside the exclusive identification of piceol (4-hydroxyacetophenone) in the latter. These aromatic, non-alkaloid phenolic compounds, characterized by a mild vanilla-like fragrance, highlight an underappreciated facet of chemical diversity within the Amaryllidaceae, traditionally known for its alkaloid constituents. The reported anti-inflammatory activity of acetovanillone aligns with the ethnobotanical use of these

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species in treating inflammatory ailments, thereby expanding the pharmacognostic value of the family to include phenolic antioxidants and underscoring their therapeutic potential. In *Scutellaria ramosissima* (Fig 4), GC-MS profiling revealed acetovanillone as a major component alongside flavonoids, hydrocarbons and sterols, suggesting potential synergistic interactions that may underlie the plant's anti-inflammatory and cytotoxic effects (Mamadalieva *et al.,* 2013). *Scutellaria ramosissima* has attracted phytochemical research interest for its flavonoids' cytotoxic effects, which inhibit the proliferation of HeLa, HepG-2 and MCF-7 human cancer cells (Mamadalieva *et al.,* 2011). These findings also pave the way for the use of acetovanillone as a chemotaxonomic marker and quality control standard in herbal formulations.

Biosynthesis *via* microbial biotransformation

The microbial transformation of ferulic acid into acetovanillone by *Streptomyces tunisiensis* DSM 42037 marks a pivotal advancement in sustainable production strategies for bioactive compounds (Slama *et al.,* 2021). This bioconversion process, involving the intermediate 4-vinyl guaiacol (4-VG), was validated through time-resolved TLC, HPLC and NMR analyses. Peak production coincided with maximal enzymatic activity and biomass accumulation. Such microbial platforms offer eco-friendly alternatives to chemical synthesis, with potential for scaling in industrial fermentation systems targeting the nutraceutical, food additive and cosmeceutical sectors. These biosynthetic routes also afford opportunities to explore metabolic engineering strategies for improved yield and substrate flexibility.

Chemical structures

- ❖ Ferulic acid: 4-hydroxy-3-methoxycinnamic acid.
- ❖ 4-Vinyl guaiacol (4-VG): 4-hydroxy-3-methoxystyrene.
- ❖ Acetovanillone: 4-hydroxy-3-methoxyacetophenone.

Detailed biosynthetic pathway

Step 1: Ferulic acid → 4-Vinyl guaiacol.
Enzyme: Ferulic acid decarboxylase.
Reaction: Non-oxidative decarboxylation.
Ferulic acid → (Decarboxylase) → 4-Vinyl guaiacol + CO_2.
Step 2: 4-Vinyl guaiacol → Acetovanillone.
Enzyme: Likely oxidase or monooxygenase.
Reaction: Oxidation and rearrangement of the vinyl side chain, producing Acetovanillone as the final aromatic ketone product.
4-Vinyl guaiacol → (Oxidase) → Acetovanillone.

Pathway summary

Ferulic acid → (Decarboxylase) → 4-Vinyl guaiacol → (Oxidase) → Acetovanillone.

Neuroprotective potential in amyotrophic lateral sclerosis (ALS)

Recent computational and genomics-based investigations have highlighted acetovanillone's ability to bind and inhibit the N-terminal domain (NTD) of the TDP-43 protein, which plays a central role in the pathogenesis of ALS (Shah and Kim, 2021). In virtual screening analyses of over 1300 aromatic compounds, acetovanillone demonstrated robust binding affinity, outperforming clinically approved agents



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Fig 1: *Boophone disticha* (Century plant or tumbleweed).



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Fig 2: *Crinum graminicola*.



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Fig 3: Sand lily (*Crinum buphanoides*).

such as Edaravone (Yoshino, 2019). It also exhibited favourable blood-brain barrier penetration, high gastrointestinal absorption and limited interaction with cytochrome P450 enzymes. Importantly, gene expression profiling indicated downregulation of ALS-linked genes (*e.g.*, CXCL2, NOP56, SOD1) and upregulation of antioxidant genes like GCLM (Glutamate-Cysteine Ligase Modifier). It plays a crucial role in the synthesis of glutathione, a major antioxidant in cells and its modifier subunit is essential for maintaining cellular redox balance and protecting against oxidative damage (Tosic *et al.,* 2006, Table 1). These findings support the compound's potential as a neuroprotective agent and warrant further preclinical validation.

Cardioprotection and oxidative stress modulation

In a model of cadmium-induced cardiotoxicity, the combined administration of acetovanillone and carvedilol resulted in significant attenuation of myocardial injury (Hassanein *et al.,* 2023). The therapeutic synergy was evident in biochemical, histopathological and molecular markers (Ho *et al.,* 2013; Priya *et al.,* 2017). Combination of Carvedilol (CV) and Acetovanillone (ACET) showed more pronounced effects in enhancing GSH and SOD levels, while reducing MDA content. Cadmium (Cd) exposure markedly elevated NADPH oxidase expression and also led to decreased mRNA expression of Nuclear Factor Erythroid 2–Related Factor 2 (Nrf2), Heme Oxygenase-1 (HO-1) and Sirtuin 1 (SIRT1), alongside increased expression of Kelch-like ECH-Associated Protein 1 (KEAP-1) and Forkhead Box O3 (FOXO-3). Treatment with combination significantly suppressed NADPH oxidase expression and enhanced Nrf2, HO-1 and SIRT1 levels (Alcendor *et al.,* 2007; Nadtochiy *et al.,* 2011), downregulating KEAP-1 and FOXO-3 (Saha *et al.,* 2020). Inflammatory cytokines like TNF-α and IL-6, along with necroptotic proteins like Receptor-Interacting Protein Kinase 1 and 3 (RIPK1, RIPK3) and Mixed Lineage Kinase Domain-Like protein (MLKL), were markedly suppressed (Amirshahrokhi and Zohouri, 2021; Abdel-Aziz *et al.,* 2022). These results underscore the potential of acetovanillone-based combination therapy in cardiovascular diseases, particularly those with environmental or occupational etiologies involving heavy metals (Table 1).

Mechanotransduction in endothelial cells

The application of fluid shear stress on bovine aortic endothelial cells revealed an increase in fluid-phase endocytosis, a process mitigated by acetovanillone through NOX inhibition (Niwa *et al.,* 2006). This pathway involves ROS-mediated activation of protein kinase C (PKC), as confirmed by kinase assays and fluorescence imaging (Table 1). Interestingly, acetovanillone selectively blocked shear-induced uptake without affecting static conditions, suggesting its potential in modulating endothelial function under biomechanical stress. These findings are relevant to oxidative stress related atherosclerosis and other vascular disorders characterized by altered shear profiles and endothelial dysfunction (Niwa *et al.,* 2006).

Therapeutic role in hypertension

Preventive effects of acetovanillone were studied on the development of hypertension in borderline hypertensive rats (BHR). The supplemented group had significant reduction in systolic blood pressure compared to untreated BHRs, while normotensive controls remained at 119±2/ mmHg (Jendeková *et al.,* 2006). Acetovanillone increased nitric oxide synthase (NOS) activity in the kidneys, but not in the left ventricle and reduced oxidative stress markers like conjugated diene levels and NF-κB expression. These changes suggest that acetovanillone helps to mitigate oxidative stress and inflammation, thereby supporting vascular health (Jendeková *et al.,* 2006). The findings support previous research linking NADPH oxidase-derived superoxide with hypertension and highlight acetovanillone's potential as a therapeutic agent for preventing the progression of high blood pressure by preserving nitric oxide bioavailability and reducing oxidative damage (Table 1).

Hepatoprotection in methotrexate-induced injury

Methotrexate (MTX)-induced hepatotoxicity is primarily mediated by oxidative stress and inflammatory pathways. Acetovanillone co-administration ameliorated liver injury by restoring antioxidant enzyme levels (SOD, CAT, GSH, GST) and reducing lipid peroxidation (Abd El-Ghafar *et al.,* 2022). It also upregulated Nrf2/ARE and SIRT1 pathways while downregulating NF-κB, STAT3 and AP-1 signalling. Histopathological studies corroborated biochemical findings, revealing near-normal liver architecture in acetovanillone-treated groups. Moreover, acetovanillone enhanced MTX's cytotoxicity against multiple cancer cell lines, reducing its effective dose (Monthakantirat *et al.,* 2005). These dual effects support its potential as a chemo protective and chemo sensitizing adjunct (Table 1).

Antiviral applications against SARS-CoV-2

Acetovanillone from *Picrorhiza kurroa* (Kandpal *et al.,* 2023, Fig 5), myrtenol from *Cyperus rotundus* (Mustak) and nimbochalcin from *Azadirachta indica* (Neem) showed



Extractives and biological activities of lamiaceae species growing in uzbekistan. Scientific figure on research gate. Available from: https://www.researchgate.net/figure/Scutellaria-ramosissima-C-A-Gaziev_fig13_333777151.

Fig 4: *Scutellaria ramosissima*. © A. Gaziev.

Table 1: Reported biological activities of acetovanillone (Apocynin) and their mechanistic insights.

Biological activity/model	Mechanistic highlights	Key findings/effects	References
Neuroprotective potential in ALS	Binding inhibition of TDP-43 N-terminal domain; modulation of oxidative stress-related genes (↑GCLM, ↓CXCL2 and NOP56)	Exhibited superior binding affinity to TDP-43 vs. Edaravone; good BBB penetration and GI absorption; supports neuroprotection and redox homeostasis	Shah and Kim, 2021; Yoshino, 2019; Tosic *et al.,* 2006
Cardioprotection in Cd-induced toxicity	Modulation of Nrf2/HO-1/SIRT1 and suppression of KEAP-1/FOXO-3/NADPH oxidase; anti-inflammatory and anti-necroptotic actions	Combined ACET + Carvedilol enhanced GSH, SOD, reduced MDA; downregulated TNF-α, IL-6, RIPK1/3, MLKL; improved cardiac histology	Hassanein *et al.,* 2023; Ho *et al.,* 2013; Priya *et al.,* 2017; Alcendor *et al.*, 2007; Nadtochiy *et al.,* 2011; Saha *et al.,* 2020; Amirshahrokhi and Zohouri, 2021; Abdel-Aziz *et al.,* 2022
Mechanotransduction in endothelial cells	NOX inhibition; attenuation of ROS-PKC signalling under shear stress	Prevented shear-induced endocytosis without affecting static uptake; potential regulator of endothelial oxidative stress	Niwa *et al.,* 2006
Antihypertensive role	↑ Renal NOS activity, ↓ NF-κB, ↓ oxidative markers (conjugated dienes); NADPH oxidase inhibition	Lowered systolic BP in borderline hypertensive rats; preserved no bioavailability	Jendeková *et al.,* 2006
Hepatoprotection in MTX-induced injury	Activation of Nrf2/ARE, SIRT1; Suppression of NF-κB, STAT3, AP-1; enhancement of antioxidant enzymes	Restored SOD, CAT, GSH, GST; Reduced MDA; Normalized hepatic structure; sensitized cancer cells to MTX	Abd El-Ghafar *et al.,* 2022; Monthakantirat *et al.,* 2005
Antiviral activity against SARS-CoV-2	Docking with 3CLpro and RdRp; Stable complex formation; anti-inflammatory and antioxidant potential	Strong binding affinity; Stable MD profiles; Good ADME profile; Candidate for COVID-19 therapy	Khuntia *et al.,* 2021
Chemoprevention in cyclophosphamide toxicity	Activation of PI3K/Akt/mTOR and Nrf2; Upregulation of cytoglobin; Antioxidant restoration	Prevented cardiac and pulmonary injury; Decreased MDA; Increased SOD, GSH	Hassanein *et al.,* 2020; Abd El-Ghafar *et al.,* 2021
Antibacterial and antileishmanial derivatives	Structural modification to benzyl ether derivatives; Inhibition of Mtb Cyp125; Antimicrobial action	Broad-spectrum antibacterial and antileishmanial effects; Favorable drug-likeness and binding affinity	Lu *et al.,* 2011; Kim *et al.,* 2012; Sammaiah *et al.,* 2015; Baker *et al.,* 2019; Calkilic *et al.,* 2022; Daina *et al.,* 2017
Role in plant immunity and redox homeostasis	Phenolic accumulation under pathogen stress; Redox modulation; H_2O_2- Dependent dimerization	Enhances phenolic response; Modulates oxidative burst without inhibiting plant NOX	Baker *et al.,* 2015; Baker *et al.,* 2018; Baker *et al.,* 2020
Antioxidant properties (Comparative)	Radical scavenging *via* phenolic structure; Para-positioned conjugated double bonds enhance activity	Ranked among 2-methoxyphenols for antioxidant potential in smoke-derived phenolics	Bortolomeazzi *et al.,* 2007

Table 1: Continue..

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Anti-inflammatory and immunomodulatory effects	NADPH oxidase inhibition; MPO-mediated activation to diapocynin; regulation of Keap-1/Nrf2/AKT and NF-κB/iNOS/TLR4	Reduced ROS, IL-6 and NF-κB; Effective in IBD, asthma, arthritis, atherosclerosis	Van den Worm *et al.*, 2001
Reproductive and cryoprotective effects	Regulation of Nrf2/AKT/SIRT1; Suppression of NF-κB/iNOS/TLR4; Inhibition of NADPH oxidase in sperm cryopreservation	Protected against MTX-induced testicular injury; Improved testosterone, sperm motility, post-thaw viability	Baker *et al.*, 2019; Sayed *et al.*, 2021; Ademola *et al.*, 2023; Kumar *et al.*, 2022
Cytotoxic and redox effects in glial cells	ROS overproduction, MDA and LDH elevation; GSH depletion; metal-ion mediated oxidation	Dose-dependent pro-oxidant effects at higher concentrations; mitigated by GSH or metal chelators	Riganti *et al.*, 2006

promising binding affinity toward the SARS-CoV-2 proteins (Khuntia *et al.,* 2021). Computational docking studies have demonstrated strong interactions of acetovanillone and myrtenol with key SARS-CoV-2 enzymes, including RNA-dependent RNA polymerase (RdRp) and the main protease 3CLpro. Molecular dynamics simulations validated the stability of these complexes. Acetovanillone exhibited desirable pharmacokinetic properties, including good gastrointestinal absorption and blood-brain barrier permeability, with minimal predicted toxicity. Given its additional anti-inflammatory properties, it may attenuate COVID-19-related cytokine storms (Khuntia *et al.,* 2021). These findings position acetovanillone as a potential candidate for antiviral drug development, particularly from phytopharmaceutical sources (Table 1).

Chemoprevention in cyclophosphamide toxicity

Cyclophosphamide (CP) is a widely used chemotherapeutic agent with significant cardiotoxic and pulmonary side effects (Hassanein *et al.*, 2020). Acetovanillone administration ameliorated CP-induced cardiac and lung injuries by restoring PI3K/Akt/mTOR signalling and enhancing Nrf2-mediated antioxidant defences (Abd El-Ghafar *et al.,* 2021). It also upregulated cytoglobin, a novel protective protein implicated in oxygen transport and ROS scavenging. Histological analyses showed preserved tissue integrity, while biochemical assays confirmed reduced MDA and increased SOD and GSH levels. These multifaceted protective mechanisms highlight acetovanillone's role in reducing chemotherapy-associated organ toxicity (Table 1).

Antibacterial and antileishmanial derivatives

Structural modification of acetovanillone (Fig 6) to form benzyl ether derivatives resulted in compounds with significant antibacterial and antileishmanial activity (Table 1). Acetovanillone and its derivatives have been documented to exhibit a range of biological activities, including anti-arthritic, anti-inflammatory, antimicrobial and antioxidant effects (Lu *et al.,* 2011; Kim *et al.,* 2012; Sammaiah *et al.,* 2015; Baker *et al.,* 2019). Compounds like 1-[4-(4-Bromobenzyl) oxy)-3-methoxyphenyl] ethanol (4a) and 1-[4-(4-Chlorobenzyl) oxy)-3-methoxyphenyl] ethanol (4b) exhibited broad-spectrum activity against Gram-positive and Gram-negative bacteria, as well as *Leishmania major* (Calkilic *et al.,* 2022). Docking studies targeting *Mycobacterium tuberculosis* Cyp125 enzyme revealed strong binding affinities, supporting its potential in tuberculosis therapy. Compounds 1-[4-Bromobenzyl) oxy] -4-[1-(4-bromobenzyl)oxy) ethyl]-2-methoxybenzene (6b), 1-[4-Bromobenzyl)oxy]-4-[1-(4-chlorobenzyl)oxy) ethyl]-2-methoxybenzene (6c), 4-[1-(4-Bromobenzyl) oxy)ethyl]-1-[4-chlorobenzyl)oxy]-2-methoxybenzene (7b) and 1-[4-Chlorobenzyl) oxy]-4-[1-(4-chlorobenzyl) oxy) ethyl]-2-methoxybenzene (7c) exhibited the highest binding affinity to the Mtb Cyp125 receptor. Physicochemical and ADME (Absorption, Distribution, Metabolism and Excretion) profiling confirmed drug-like characteristics, while

elemental and spectroscopic analyses ensured compound purity (Daina *et al.,* 2017). These findings suggest the viability of acetovanillone scaffolds in antimicrobial drug discovery.

Role in plant immunity and redox homeostasis

In plant systems, acetovanillone accumulates in response to bacterial invasion and participates in the oxidative burst within the leaf apoplast (Baker *et al.,* 2018). Unlike its mammalian role, it does not inhibit plant NOX enzymes but modulates redox status through its oxidative products (Table 1). These effects are pH-dependent and influenced by hydrogen peroxide levels, which promote acetovanillone dimerization (Baker *et al.,* 2015). Its presence enhances the overall phenolic profile during pathogen attack, although its precise role in signalling remains to be elucidated. This dual functionality in both plant and animal systems underscores its ecological and pharmacological versatility (Baker *et al.,* 2020).

Antioxidant potential of major phenolic compounds found in wood smoke and smoke flavourings used in the food industry were evaluated by employing crocin bleaching inhibition, DPPH radical scavenging and oxidation potential assays (Bortolomeazzi *et al.,* 2007). Among the tested compounds, isoeugenol stood out for its potent radical scavenging capacity, underscoring the importance of para-positioned conjugated double bonds in enhancing antioxidant behaviour (Vikram *et al.,* 2024). The study provides a comparative ranking of antioxidant efficiency *i.e.* dihydroxybenzenes followed by 2,6-dimethoxyphenols and 2-methoxyphenols (Bortolomeazzi *et al.,* 2007).

Potential candidate in oxidative stress-related inflammatory disorders

Apocynin as a lignin degradation product in the wood and paper industry has emerged as a promising non-steroidal anti-inflammatory agent due to its ability to inhibit ROS production in activated neutrophils by interfering with NADPH oxidase assembly (Van den Worm *et al.,* 2001). Apocynin is converted into its active form by myeloperoxidase (MPO) in the presence of ROS from activated PMNs. One proposed active metabolite is diapocynin, a dimer of apocynin, inhibit NADPH oxidase-dependent ROS production. Studies suggest it selectively targets activated neutrophils, showing therapeutic promise in conditions such as chronic inflammation, inflammatory bowel disease (IBD), asthma and atherosclerosis (Van den Worm, 2001). In IBD models, oral administration of apocynin markedly reduced jejunal damage and histopathological changes. Its anti-asthmatic efficacy has been demonstrated in OVA- and PAF-induced animal models, significantly reducing bronchoconstriction without typical bronchodilator mechanisms. Similarly, in atherosclerosis research, apocynin inhibited thrombin-stimulated ROS production in endothelial cells, highlighting a novel approach to vascular protection by targeting endothelial NADPH oxidase. Apocynin treatment has also shown sustained anti-inflammatory effects in arthritis models, notably by reducing joint swelling in a rat model of rheumatoid arthritis, reducing IL-6 levels while sparing chondrocyte metabolism. Additionally, apocynin modulates key inflammatory mediators by reducing iNOS, NF-κB and TLR4 expression and regulating Keap-1/Nrf2/AKT signalling pathways (Van den Worm *et al.,* 2001). Despite conflicting evidence on nitric oxide production in human neutrophils, apocynin's role as a selective respiratory burst inhibitor remains well-supported by *in vitro, in vivo* and *in silico* studies (Table 1).

Reproductive biology and cryopreservation

Apocynin (APO) demonstrates significant protective effects against MTX-induced testicular toxicity, a growing concern due to the widespread clinical use of MTX in cancer and autoimmune disease treatment (Baker *et al.,* 2019). MTX alone is associated with reduced reproductive hormone levels and severe histopathological alterations in the testes (Sayed *et al.,* 2021). However, co-administration with APO markedly restored testicular structure and function, evidenced by elevated serum testosterone, FSH and LH levels. Mechanistically, APO enhanced the antioxidant



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Fig 5: *Picrorhiza kurroa* (Kutki).

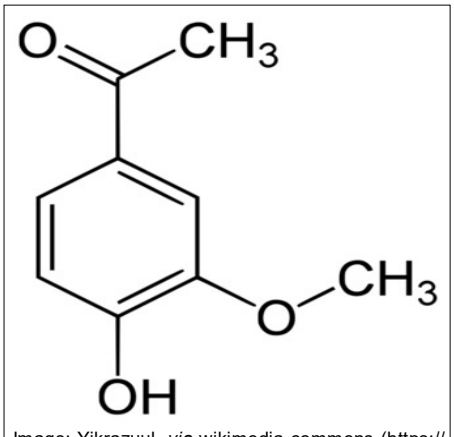


Image: Yikrazuul, *via* wikimedia commons (https://commons.wikimedia.org/w/index.php?curid=7738511).

Fig 6: Chemical structure of acetovanillone, also known as apocynin.

defence system through the upregulation of Nrf2, cytoglobin, PPAR-γ, SIRT1, AKT and phosphorylated AKT, while suppressing Keap-1 expression. In parallel, APO significantly mitigated inflammation by down-regulating the expression of pro-inflammatory mediators including NF-êB-p65, iNOS and TLR4. These findings were further validated through *In-silico* modelling and network pharmacology analysis, which confirmed the modulation of these key signalling pathways. Collectively, the data highlight APO's ability to abrogate MTX-induced testicular damage by regulating the Keap-1/Nrf2/AKT axis and suppressing the iNOS/NF-κB/TLR4 inflammatory pathway, supporting its potential for clinical application in preserving male reproductive health during MTX therapy (Sayed *et al.,* 2021).

One study investigated the influence of NADPH oxidase inhibition on cavernosa tissue contractile activity by incubating isolated tissue in acetovanillone, which may have implications for erectile function. Additionally, the antioxidant status was evaluated to explore the therapeutic potential of the ethanol extract of *Tridax procumbens* leaves (EETP) in ameliorating erectile dysfunction and reproductive impairments caused by chronic variable stress (CVS) in male Wistar rats (Ademola *et al.,* 2023). EETP supplementation during CVS exposure significantly improved sperm motility, reduced abnormal spermatozoa and enhanced the contractile and relaxation responses of the corpus cavernosa, particularly through acetylcholine and nitric oxide-mediated pathways. EETP also attenuated calcium- and potassium-mediated contractile impairments, indicating a restoration of key physiological mechanisms involved in erectile function. The extract exhibited notable antioxidant effects, as evidenced by reduced serum MDA levels and elevated SOD activity, comparable to vitamin C supplementation (Ademola *et al.,* 2023). These antioxidative effects are attributed to the polyphenolic and flavonoid content of EETP (Table 1). Moreover, EETP supplementation resulted in lower cortisol levels and elevated testosterone levels, suggesting modulation of stress and hormonal balance likely aided by its phytosterol constituents.

Cryopreservation-induced oxidative stress significantly impairs sperm quality. Supplementation of Tris-based extenders with Mito TEMPO and Acetovanillone improved post-thaw motility, viability and membrane integrity in buffalo spermatozoa (Kumar *et al.,* 2022; Srivastava *et al.,* 2024). The combined use of a mitochondrial ROS scavenger and a NOX inhibitor reduced MDA levels and elevated antioxidant enzymes (SOD, CAT, GPx). This dual-targeted approach offers a novel strategy for enhancing artificial insemination outcomes, particularly in species sensitive to cryodamage (Table 1).

Cytotoxic and redox effects in glial cells

Contrary to its antioxidant reputation, Acetovanillone exhibits pro-oxidant and cytotoxic effects in murine glial N11 cells at higher concentrations (Riganti *et al.,* 2006). Elevated levels of MDA, lactate dehydrogenase (LDH) and H_2O_2, along with GSH depletion, confirmed the induction of oxidative stress. *In vitro* models indicated that metal ion interactions, particularly with copper, enhance ROS generation and thiol oxidation. Supplementation with GSH or chelation with nitrilotriacetic acid (NTA) mitigated these effects (Riganti *et al.,* 2006). These findings are critical in contextualizing dosage strategies and underscore the importance of cellular context in interpreting Acetovanillone's redox behaviour (Table 1).

Conclusion and Future Perspectives

Acetovanillone represents a pharmacologically versatile compound, effective across a spectrum of oxidative stress-related pathologies, including neurodegeneration, inflammation, cardiotoxicity and microbial infections. Its dualistic redox behaviour necessitates cautious therapeutic application, yet its broad-spectrum efficacy underscores its promise in integrative medicine and biotechnology. Future research should focus on comprehensive *in vivo* studies, formulation development and clinical trials. Additionally, structure-activity relationship (SAR) studies and metabolic pathway elucidation will facilitate the design of more potent and selective acetovanillone-based therapeutics. The integration of systems biology, omics technologies and AI-driven drug discovery platforms can further accelerate its translational potential.

Conflict of interest

The author declares no conflict of interest.

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