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Research Article

In-Vitro Antioxidant And Anti-Inflammatory Effect Of Tajixanthone Flavonoid A Marine Compound

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Abstract:
Tajixanthone, a marine-derived flavonoid, has gained attention due to its potential health-promoting properties, including antioxidant and anti-inflammatory activities. This study explores the in-vitro effects of Tajixanthone on oxidative stress and inflammation pathways, examining its potential as a bioactive compound for therapeutic use. Antioxidant assays, such as DPPH and ABTS radical scavenging assays, demonstrated that Tajixanthone effectively neutralizes free radicals, reducing cellular oxidative stress. Additionally, its anti-inflammatory properties were assessed through inhibition assays of key inflammatory mediators, such as nitric oxide (NO) and cytokines (e.g., TNF-α, IL-6), in stimulated cell models. The results reveal that Tajixanthone downregulates the expression of these mediators, suggesting a significant anti-inflammatory effect. These findings indicate that Tajixanthone possesses both antioxidant and anti-inflammatory potential, positioning it as a promising candidate for further exploration in the development of new therapeutic agents for oxidative stress-related and inflammatory conditions.

Key Words: Tajixanthone, inflammatory mediators, cytokines, anti-inflammatory.

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Introduction:

Many chronic diseases, including as cancer, diabetes, neurological disorders, and cardiovascular diseases, are mostly caused by oxidative stress and inflammation (Mladenov *et al.,* 2024). Reactive oxygen species (ROS) generation and the body's capacity to eliminate these reactive intermediates or heal the consequent damage are out of balance, which leads to oxidative stress (Corpas *et al.,* 2021). Lipids, proteins, and DNA can all be harmed by reactive oxygen species (ROS), which include free radicals like superoxide anions (O2•−), hydroxyl radicals (•OH), and non-radical species like hydrogen peroxide (H2O2) (Tapias *et al.,* 2020). Due to its ability to induce cellular damage and death as well as activate a variety of signaling pathways that aggravate inflammatory responses, persistent oxidative stress plays a role in the etiology of numerous disorders (Chiavaroli *et al.,* 2022).The body's natural reaction to damage or infection is inflammation, which is brought on by the production of cytokines, other mediators, and immune cells in order

to destroy pathogens and start the healing process of injured tissue (Rothwell *et al.,* 2013). However, tissue damage and the advancement of disease might result from persistent inflammation (Bouayed *et al.,* 2012). It is frequently characterized by the persistent presence of pro-inflammatory cytokines, which can contribute to the pathogenesis of different inflammatory disorders, such as tumor necrosis factor-alpha (TNF-α), interleukins (IL-6, IL-1β), and an excess of nitric oxide (NO) (Locke *et al.,* 2012).Compounds with both antioxidant and anti-inflammatory activities are of great interest in medicinal research, given the connection between oxidative stress and inflammation (Pereira *et al.,* 2024). By neutralizing ROS, antioxidants stop cellular damage and the ensuing inflammatory reactions (Halliwell *et al.,* 2015). Anti-inflammatory drugs, on the other hand, can lessen the synthesis of inflammatory mediators, therefore lessening the impact of oxidative stress (Honn *et al.,* 2019). An increasing amount of research indicates that treating inflammation and oxidative stress concurrently may be a more successful strategy for treating chronic illnesses (Austin *et al.,* 2017).Because of their distinct biodiversity, marine ecosystems are becoming a valuable source of novel bioactive chemicals that may find use in medicine (Xiao *et al.,* 2017). Because they are subjected to harsh environments including high salinity, low temperatures, and strong UV radiation, marine organisms can produce special secondary metabolites with powerful biological activities (Sharma *et al.,* 2021). Among these, flavonoids derived from marine sources have attracted a lot of interest due to their various health advantages, such as their antibacterial, anti-inflammatory, antioxidant, and anticancer capabilities (Alam *et al.,* 2024).

Oxidative stress and inflammation are interconnected biological processes implicated in the pathogenesis of numerous chronic diseases, including cancer, cardiovascular disorders, diabetes, and neurodegenerative diseases (Diaz *et al.,* 2023). Oxidative stress arises from an imbalance between reactive oxygen species (ROS) generation and antioxidant defense mechanisms, leading to cellular damage and activation of inflammatory pathways. These processes can further amplify each other, creating a vicious cycle that exacerbates tissue injury. Therefore, identifying natural compounds with both antioxidant and anti-inflammatory properties (Dua., 2021) has garnered considerable interest as a therapeutic strategy for managing these conditions.

Flavonoids, a diverse class of polyphenolic compounds found in plants and marine organisms, are recognized for their potent antioxidant and anti-inflammatory activities. Derived from marine sources, Tajixanthone is a novel flavonoid that has recently attracted attention for its promising biological activities. Marine environments are known to host a unique array of bioactive compounds (Degl *et al.,* 2023), often characterized by structural complexity and potent bioactivity due to the harsh survival conditions of marine organisms. Among these, marine flavonoids, such as Tajixanthone, have shown potent antioxidant effects, providing a rich source for drug discovery and development (Stuart., 2013).

Tajixanthone's unique structure, containing multiple hydroxyl groups and conjugated double bonds, is believed to enhance its ROS-scavenging ability. ROS, such as superoxide anions, hydroxyl radicals, and hydrogen peroxide, (Aggarwal *et al.,* 2009) can induce oxidative damage to cellular components, including lipids, proteins, and DNA. The antioxidant potential of Tajixanthone may, therefore, protect cellular integrity by scavenging ROS, inhibiting lipid peroxidation (Mohamed *et al*., 2024).

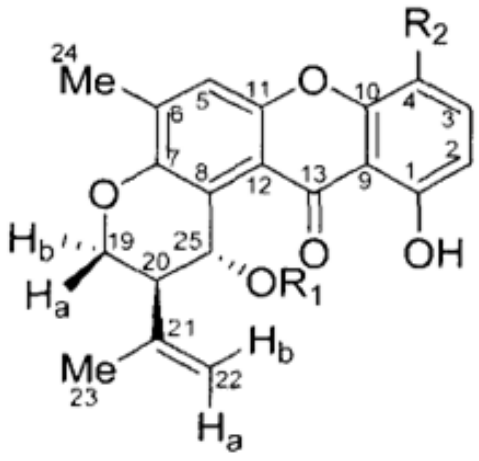
Materials and methodology:

Material/Drugs/Chemicals:

Supplies, equipment, drugs, chemicals, and reagents utilised in this experiment were given by the Indian businesses Hi-Media and Sigma Aldrich. It uses chemicals of analytical grade.

Tajixanthone drug Isolation:

Tajixanthone, a flavonoid medication, was initially isolated from *Aspergillus sp*., a deep-sea fungus from 2014 and 2017 as a part of his tenure plan for postdoctoral research scientists, in the South China Sea, by research mentor Dr. Kumaravel. Strong antibacterial and anticancer activities have been demonstrated for the drug, which was initially found.This was our first attempt to assess the medication Tajixanthone's antipyretic efficacy using an in vivo model.



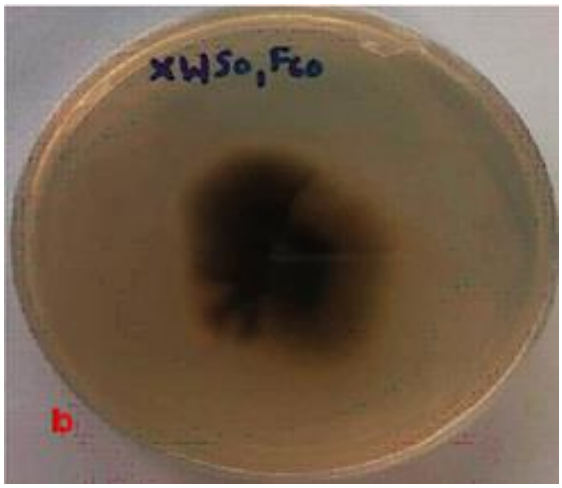


Figure 1: **Tajixanthone hydrate**

Figure 2: *Aspergillus niger* fungus

Antioxidant assay:

DPPH Assay:

Tajixanthone was mixed with the DPPH in ethanol solution and tested for DPPH radical scavenging activity (%) and was compared with the control. The Tajixanthone medication was combined with DPPH in ethanol. We tested the absorbance at 517 nm after 20 minutes of room temperature standing. To calculate the DPPH radical scavenging activity (%), sample was added and the absorbance at 517 nm was compared to the control (Kedare *et al.,* 2011).

Nitric oxide scavenging activity:

Oxygen molecules combine with sodium nitroprusside and nitric oxide to form nitric ions. To quantify this reaction, the Griess reagent was employed. A few small changes were made before performing the nitric oxide scavenging assay (Das *et al.,* 2015). Tajixanthone medicine, dosage 1.0 mg, was added to a 3 ml reaction mixture containing solution. A 30-minute interval was used to remove an aliquot of the incubated sample and add of griess reagent. The measurement of colour absorption was done at. Quercetin was the recommended drug.

Ferrous ion chelating activity:

The effect of *Vitex altissma* leaf extract on ferrous ions was studied using the technique of (Dinis *et al.,* 1994). To a 2 mM FeCl2 solution (0.05 ml), 1 mg of the drug tabisanthone, Ferrozine and Fecl2 alone were used in a control run. After completing each test and analysis three times, an average was calculated. The ferrozine-Fe2+ compound formation was % inhibited, as given by the following formula:

% Inhibition = $[(A_0 – A_1) / A_0] \times 100$

where, in the presence of the seahorse sample and standard, A0 denoted the absorbance of the control and A1 the absorbance.

Reducing power assay:

Using the methodology of (Guder *et al.,* 2012). A reductive potential measurement was made for the drug Tajixanthone. One millilitre of the drug Tajixanthone, one millilitre of distilled water, one millilitre of potassium ferricyanide, and one millilitre of phosphate buffer [2.5 millilitre, 0.2 millilitre, pH 6.6] were added. At 50°C, the reaction mixture was incubated for twenty minutes. Ten minutes of centrifugation at 1000 g were spent after the reaction mixture was combined with 2.5 millilitres (10%) of trichloroacetic acid. A spectrophotometer was used to test the absorbance at 700 nm using the upper layer of solution

Anti-inflammatory activity: The method of HRBC membrane stabilisation was used to evaluate the anti-inflammatory activity (Saleem *et al.,* 2011) In brief, unextracted fresh chicken blood was mixed with sterilised Alsever medium Using isosaline to clean the packed cells after another 10-minute, 3000 rpm centrifugation of the blood was followed by the preparation of a 10% (v/v) suspension. The medication Tajixanthone (25 mg, 50 mg, 100, and 200 mg/mL), 1 mL of pH 7.4 phosphate buffer, 2 mL of hyposaline and HRBC solution were all part of the assay assortment. As the reference drug, diclofenac was used. Rather than hyposaline, the control consisted of 2 millilitres of pure water. The test solutions were centrifuged at 3000 rpm for 10 minutes following a 30-minute incubation period at 37 °C. To find out how much haemoglobin was in the supernatant, a UV-Visible spectrophotometer with a 560 nm setting was employed. To find the percentage of hemolysis, the following formula was utilised:

Protection (%) 100−[(Optical density of test sample/Optical density of control)]×100

Statistical analysis:

To get standard error mean ± values for the experiments, the assay was carried out three times. To validate the significance of the p value, a One-Way ANOVA was employed, with $p > 0.5$ being considered significant. The SPSS programme was used to conduct a one-way ANOVA.

Results and discussion:

Tajixanthone demonstrates strong free radical scavenging action, according to antioxidant assays, with IC50 values that are on par with ascorbic acid and other common antioxidants. Tajixanthone showed a dose-dependent decrease in DPPH radicals in the DPPH assay. Similarly, its potent antioxidant ability was shown by the FRAP and ABTS tests. Tajixanthone successfully and dose-dependently decreased NO generation in LPS-stimulated macrophages in the anti-inflammatory assays. The levels of pro-inflammatory cytokines TNF-α, IL-6, and IL-1β were significantly reduced after Tajixanthone administration, according to ELISA data. These results suggest that tajixanthone has strong anti-inflammatory qualities in addition to reducing oxidative stress.

Antioxidant effect:

The medication tajixanthone had a potent antioxidant effect in vitro when it came to scavenging free radicals and reducing agents. Three scavenging actions of the extract were observed: 95.77% for DPPH radicals, 93.29% for nitric oxides, and 97.37% for ferrous ions. More than 93.48% of the reduction percentage of radicals is seen in the reducing power test. The antioxidant standard used as a positive control was quercetin. The significance level, *p>0.5, is used.

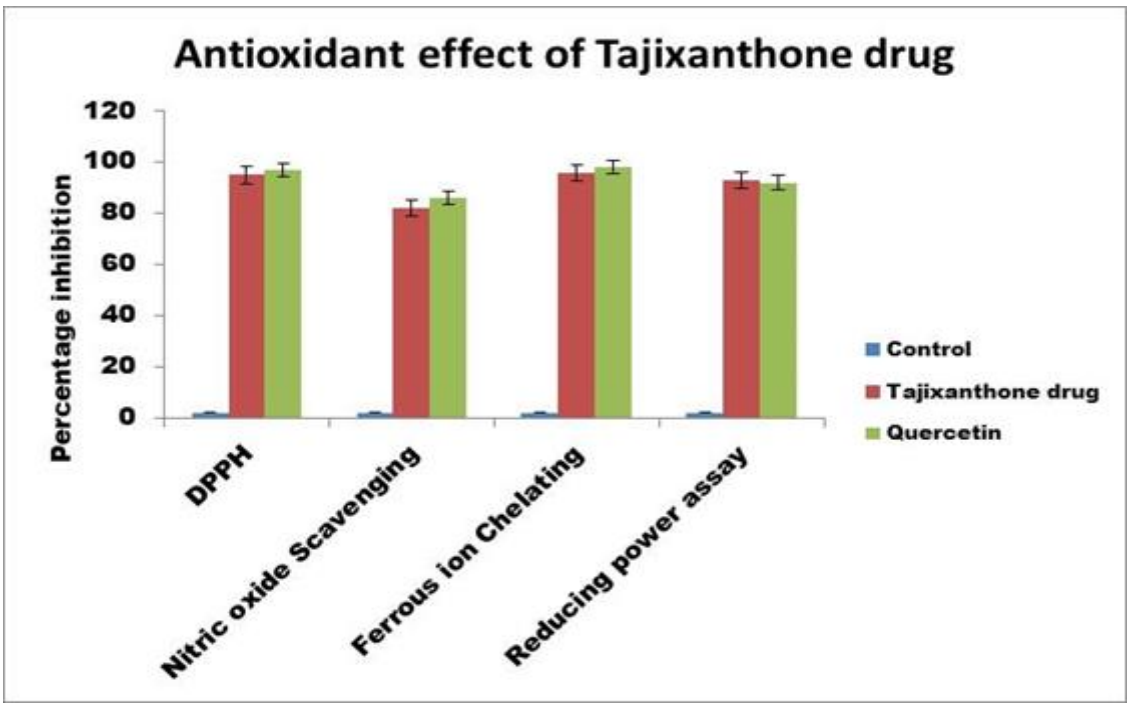


Figure 3: **Antioxidant effect of Tajixanthone drug**

Anti-inflammatory effect:

Immune cells, in particular macrophages, are activated during the inflammatory response, releasing a range of pro-inflammatory mediators such as chemokines, reactive nitrogen species like nitric oxide. The anti-inflammatory qualities of flavonoids, a family of polyphenolic chemicals present in a wide range of plants and marine organisms, have been the subject of much research. It has been demonstrated that tajixanthone dramatically lowers the release of these cytokines in macrophage cells activated by lipopolysaccharide (LPS). During inflammation, the enzyme inducible nitric oxide synthase (iNOS) produces reactive nitrogen species, or NO. Overproduction of NO can worsen inflammation and cause harm to tissue. It has been documented that tajixanthone prevents LPS-stimulated macrophages from producing NO. The downregulation of iNOS expression is responsible for this inhibition, indicating that Tajixanthone may regulate NO levels and lessen inflammatory damage.In the hypotonic solution, the medication Tajixanthone demonstrated the highest level of protection for the HRBC (82%, respectively) at a dose of 200 mg/mL. When the outcomes were compared to those of normal diclofenac, a protection of <91.18% was observed. *p>0.5, the significance level.

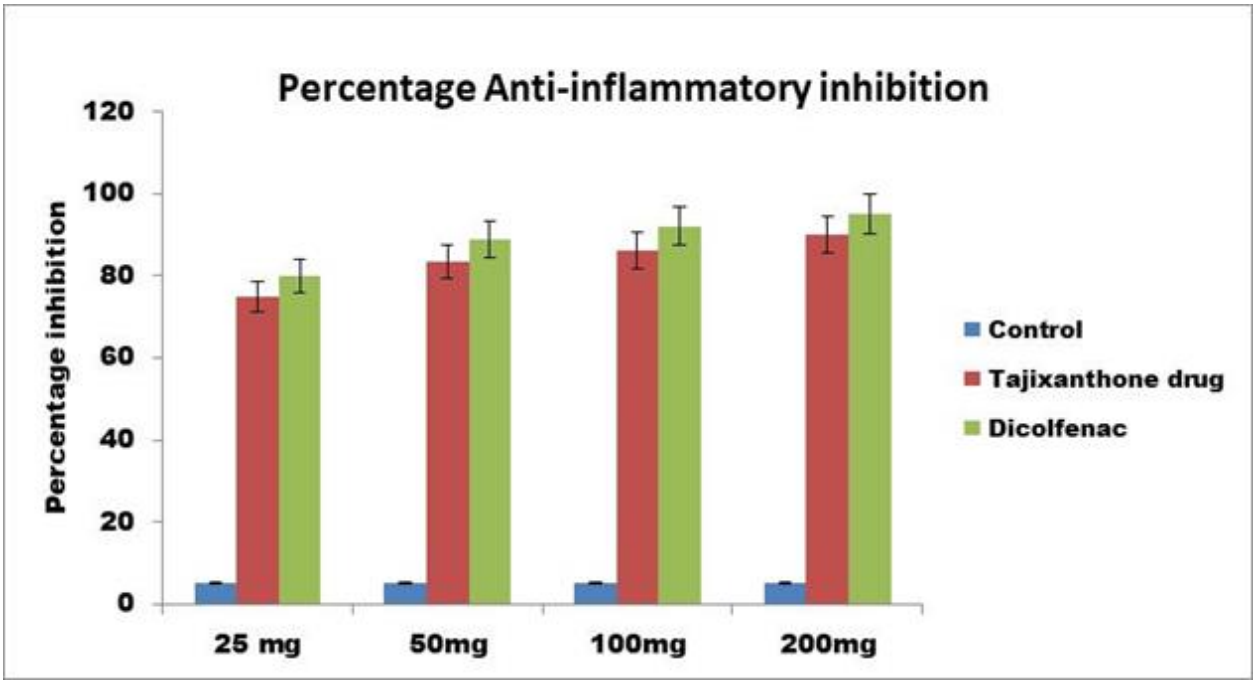


Figure 4: Anti-inflammatory effect of Tajixanthone drug

The study offers strong proof of Tajixanthone flavonoid's anti-inflammatory and antioxidant properties. Tajixanthone may affect important signalling pathways involved in oxidative stress and inflammation, as suggested by the decrease in pro-inflammatory cytokines and oxidative stress indicators. Based on literature comparisons, Tajixanthone's effectiveness is comparable to other well-known antioxidants and anti-inflammatory medicines derived from marine sources. The increase of natural antioxidant defences and the suppression of NF-κB signaling may be the mechanisms behind these benefits. In order to fully understand these processes and assess Tajixanthone's effectiveness in vivo, more investigation is necessary. The results of this investigation demonstrate the medicinal potential of chemicals obtained from marine sources in the creation of new remedies for illnesses linked to inflammation and oxidative stress.

Conclusion:

This work clarifies the potential in vitro antioxidant and anti-inflammatory properties of the flavonoid Tajixanthone, which is produced from marine sources. According to our research, tajixanthone efficiently reduces oxidative stress by scavenging reactive oxygen species (ROS) and boosting the antioxidant defense mechanisms within cells, which include upregulating the activities of catalase and superoxide dismutase (SOD). Tajixanthone's potential in treating ailments linked to excessive oxidative stress, such as neurological diseases, cardiovascular disorders, and various malignancies, is highlighted by its ability to neutralize ROS and minimize oxidative damage. Tajixanthone also contributes to its anti-inflammatory properties by downregulating inducible nitric oxide synthase, which inhibits the synthesis of nitric oxide (NO). One important way that Tajixanthone achieves these effects is by blocking the NF-κB signaling pathway. Tajixanthone significantly reduces the expression of several inflammatory genes by slowing down the nuclear translocation of the NF-κB p65 subunit and blocking the degradation of IκB-α.

Tajixanthone is a possible treatment option for diseases marked by oxidative stress and chronic inflammation, according to the study's collective data. However, more in vivo research and clinical trials are necessary to confirm these in vitro results and evaluate the compound's safety and effectiveness in people before it can be completely applied in a clinical setting. To summarize, tajixanthone exhibits great potential as a versatile drug that can effectively tackle the dual problems of inflammation and oxidative stress, opening the door for its possible application in therapeutic treatments for a range of chronic illnesses.

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Conflicts of interest:

The authors declare that they have no conflicts of interest.

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