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# The Assessment of *Equisetum* and *Syzygium cumini* comparative and combined Antioxidant potential

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**Abstract**

This study was carried out to investigate the antioxidant potential of methanolic extracts from leaves of *Equisetum* and *Syzygium cumini*. The methanolic extract of *equisetum* displayed antioxidant activity using a DPPH free radical scavenging assay. The extract indicated *Equisetum* in doze dependant manner and revealed maximum scavenging activity (88%) at a concentration of 1000µg/ml. The methanolic extract of *Syzygium cumini (*leaves) also displayed antioxidant activity using a DPPH free radical scavenging assay. The extract indicated *Syzygium cumini* activity in doze dependant manner and revealed maximum scavenging activity (88%) at a concentration of 1000µg/ml. The combine methanolic extract of *Equisetum* and *Syzygium cumini* displayed better antioxidant activity using DPPH free radical scavenging assay, 19%, 66%, and 92% at the concentrations of 100, 500, and 1000µg/mg respectively.

**Key Words:** *Syzygium cumini*, *Equisetum,* Methanolic extract, Radical scavenging, Antioxidant activity

**Introduction:**

Consumption of fruits and vegetables is shown to lower the risk for chronic diseases such as cancer, cardiovascular diseases and stroke (Yeum *et al.*, 2003). The positive health effects may be due to high contents of certain phenolic compounds in plant-derived foods (You, Duane *et al.*, 2007). Recently, phytochemicals and their effects on human health have been intensively studied. In particular, a search for antioxidants, hypoglycemic agents, and anticancer agents in vegetables, fruits, teas, spices and medicinal herbs has attracted great attention.

### 1.1. Cancer

Cancers are the collection of diseases caused by the abnormal growth and division of cells. When cancer spreads to a different body part from where it started this stage is known as metastasis. Two factors are involved in cancer cause e.g. external and internal factors. There are so many external factors in which some of them are tobacco, chemicals, radiations and infectious etc., while the internal factors include inherited mutations, hormones, immune conditions and random mutations etc. Cancer's causes are very diverse and complex and are not understood completely. There are too much different factors that lead to an increased the risk of cancer including dietary factors, certain infections, lack of physical activity, obesity and environmental pollutants (Khan, S.*et al.,* 2023)

### 1.2. Free radicals

Free radicals are produced by redox reactions when oxygen is used to produce energy in the cell. Free radicals form a group of Reactive Oxygen Species (ROS) like hydroxyl radicals, hydrogen peroxide, and superoxide anions (Munteanu & Apetrei 2021). The lower concentration of reactive oxygen species generally in favor of cellular responses and immune proper function, while the higher concentration of reactive oxygen species are dangerous to the body. The higher concentration of free radicals in the body leads to the oxidative stress that has potential of damaging the cell structure such as protein, lipid, and DNA (Pham *et al.,* 2008). This higher concentration of free radicals plays an important role in the development of degenerative and chronic diseases such as rheumatoid arthritis, autoimmune disorders, cataracts, aging, cancer, and cardiovascular and neurodegenerative diseases (Khan, S. *et al.,* 2023).

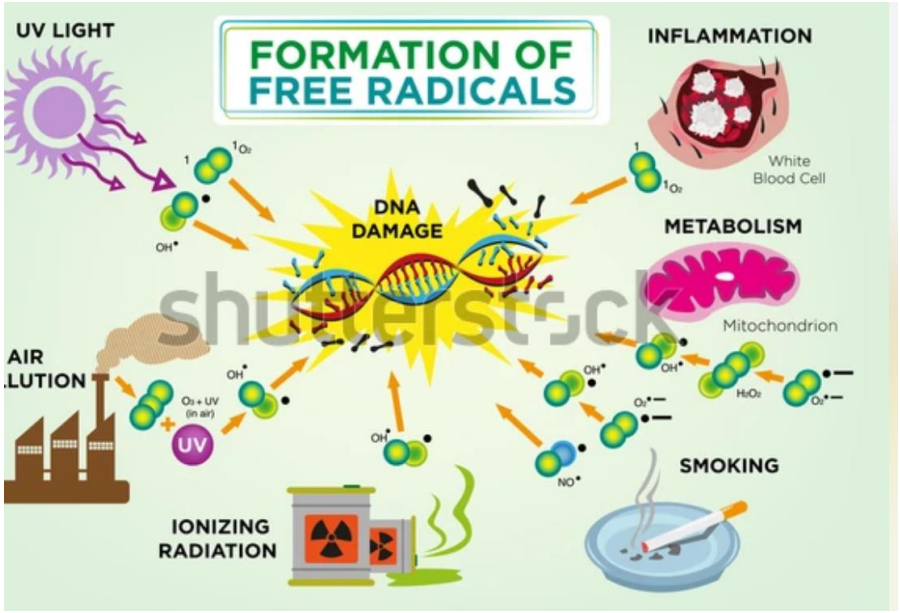



Figure 01: Formation of Free Radicals

**Table 1. List of ROS Produced during Metabolism**

| Free Radical | Symbol | Half Life |
|---|---|---|
| Radical | | |
| Superoxide | $O_2^-$ | $10^{-6}$s |
| Hydroxyl | OH | $10^{-6}$s |
| Alkoxyl radical | RO | $10^{-6}$s |
| Perxoyl Radical | ROO | 17s |
| Non Radicals | | |
| Hydrogen Peroxide | $H_2O_2$ | Stable |
| Singlet Oxygen | $^1O_2$ | $10^{-6}$s |
| Ozone | $O_3$ | s |
| Organic Peroxide | ROOH | Stable |
| Hypochlorous acid | HOCL | Stable (min) |
| Hypobromous acid | HOBr | Stable (min) |

**Table 2. Lists of RNS Produced during Metabolism**

| Free Radical | Symbol | Half Life |
|---|---|---|
| Radical | | |
| Nitric oxide | NO | s[a] |
| Nitrogen dioxide | $NO_2$ | s |
| Non Radicals | | |
| Peroxynitrite | $ONOO^-$ | $10^{-3}$ s |
| Nitrosyl cation | $NO^+$ | s |
| Nitroxyl anion | $NO^-$ | s |
| Dinirogen trioxide | $N_2O_3$ | s |
| Dinitrogen tetraoxide | $N_2O_4$ | s |
| Nitrous acid | $HNO_2$ | s |
| Peroxynitrous acid | ONOOH | Fairly stable |
| Nitryl Chloride | $NO_2Cl$ | s |

### 1.3. Antioxidant

Antioxidants are free radical scavengers that reduce the damage caused by reactive oxygen species thus they improve the immune system response and decrease the risk of degenerative diseases. Plant sources contain a variety of antioxidants (Amorati and Valgimigli 2018).Antioxidants either occur naturally or artificially provided by food or supplements (Banerjee *et al.,* 2005).Although the use of artificial antioxidants are extensive in different products like food products and medicine they may instable and can act as carcinogens therefore the interest in using natural antioxidants has been increased (Khan, S. *et al.,* 2023).

### 1.4. *Syzygium cumini:*

*S. cumini* (L.) Skeels has been attributed in the Indian folklore medicine system to possess several medicinal properties (Warrier *et al.,* 1996). The bark of the plant is astringent, sweet, refrigerant, carminative, diuretic, digestive, antihelminthic, febrifuge, constipating, stomachic and antibacterial. The fruits and seeds are used to treat diabetes, pharyngitis, spleenopathy, urethrorrhea and ringworm infection. The leaves have been extensively used to treat diabetes, constipation (Bhandary *et al.,* 1995), leucorrhoea, stomachalgia, fever, gastropathy, strangury and dermopathy (Warrier *et al.,* 1996) ), and to inhibit blood discharges in the faeces (Bhandary *et al.,* 1995). The plant possesses acetyl oleanolic acid, triterpenoids, ellagic acid, isoquercitin, quercetin, kaempferol and myricetin in different concentrations (Rastogi *et al.,* 1990). Most of these compounds have been reported to possess antioxidant and free radical scavenging activities (Tanaka *et al.,*

1998). The chemical composition and antioxidant activity of *S. cumini* fruits have been studied recently (Banerjee *et al.*, 2005, Benherlal et al 2007), but there is scant information about the antioxidant activity of *S. cumini* leaves. In this study, the total phenolic content of *S. cumini* leaves was determined, and their antioxidant properties were also evaluated.

*Syzygium cumini* trees start flowering from March to April. The flowers are fragrant and small, about 5 mm (0.2 in) in diameter. The fruits develop by May or June and resemble large berries; the fruit of *Syzygium* species is described as "drupaceous". The fruit is oblong, ovoid. Unripe fruit looks green. As it matures, its color changes to pink, then to shining crimson red, and finally to black color. A variant of the tree produces white-colored fruit. The fruit has a combination of sweet, mildly sour, and astringent flavor and tends to color the tongue purple (Julia 1987).



Figure 02: *Syzygium cumini* leaves

### 1.5 Therapeutic uses of *Syzygium cumini*

Before the discovery of insulin, various therapeutic agents were utilized for managing diabetes mellitus (DM), with numerous plants displaying antidiabetic properties. Despite the availability of different oral hypoglycemic agents and insulin for diabetes treatment, traditional healers worldwide heavily rely on medicinal plants and herbs. These natural remedies have been utilized in folk medicine for centuries, offering advantages such as minimal side effects and protection of β-cells, along with stabilizing glucose levels. Ayurveda, the traditional Indian medical system, utilizes several antidiabetic plants without known side effects. Many plants, including *Syzygium cumini* (L.) Skeels, commonly known as jambolan, have shown promising antidiabetic activity in clinical and experimental studies. While various pharmacological actions of

jambolan have been studied, including its hypoglycemic effects, a comprehensive review focusing solely on its antidiabetic efficacy is lacking, despite the abundance of literature on the subject.

*Syzygium cumini* (L.) Skeels. (*E. jambolana* Lam., MyrtaceaeThroughout many centuries, jambolan, known by various names such as black plum, java plum, and Indian blackberry, has been widely utilized by traditional healers for treating diabetes. Clinical and experimental investigations have highlighted the potential antidiabetic properties of different parts of the jambolan plant, particularly its fruits, seeds, and stem bark. Although Sagrawat et al. conducted a review on the pharmacological actions and chemical constituents of jambolan, little emphasis was placed on its antidiabetic effectiveness. Various extracts of jambolan exhibit a wide array of pharmacological effects, including antibacterial, antifungal, anti-inflammatory, and antioxidant activities, among others. Despite numerous reports in the literature, a comprehensive review specifically focusing on the antidiabetic efficacy ofjambolan is yet to be compiled (Ayyanar, M *et al.,* 2013).

### 1.6. *Equisetum*

*Equisetum* horsetail, marestail, snake grass, puzzle grass is the only living genus in Equisetaceae, a family of vascular plants that reproduce by spores rather than seeds. *Equisetum* species also have small microphyllous leaves that are arranged in true whorls (Rustishauser, 1999) and the leaves of each whorl are fused together to form a cylindrical sheath around each node (Hauke, 1993 ). Some, but not all, species form whorls of lateral branches at the nodes of the aerial stems ( Hauke, 1993 ). Unlike many other vascular plants (such as gymnosperms, angiosperms and some ferns) which produce branches in the axils of leaves, the leaves of *Equisetum* alternate with branches at each node ( Scagel *et al.,* 1984 ). Like other vascular plants, *Equisetum* produce new branches and leaves from the apical meristem. However, most of the stem lengthening is produced by intercalary meristems above each node and this produces rapid lengthening of the stem ( Stewart and Rothwell, 1993 ).

*Equisetum* is an ancient genus and comprises the sole surviving representatives of the class *Sphenopsida* (the only class of the subdivision *Sphenophytina*) ( Scagel *et al.,* 1984 ). Sphenopsids first appeared in the fossil record of the late Devonian. The earliest unequivocal sphenopsid that has been discovered is *Pseudobornia ursina*, a monopodial arborescent clonal plant of the upper Devonian which grew up to 20 m tall with stems up to 60 cm thick (Stewart and Rothwell, 1993 ; Scagel *et al.,* 1984 )

Figure 03: *Equisetum* horsetail plant



The present day species of the genus *Equisetum* are divided into two distinct subgenera: subgenus *Equisetum* , with eight species and subgenus *Hippochaete*, with seven species. There are several primary differences between the two subgenera. Species in subgenus *Equisetum* have stomata that are flush with the epidermal surface, whereas members of the subgenus *Hippochaete* have stomata that are sunken below the epidermal surface. The stems of the subgenus *Equisetum* are short-lived, relatively soft, and tend to be regularly branched, whereas the stems of the subgenus *Hippochaete* , with few exceptions, tend to be long-lived, hard, fibrous, and unbranched or irregularly branched ( Hauke, 1960 ). In addition, four of the species of the subgenus *Equisetum* demonstrate stem dimorphism between non-photosynthetic, unbranched, coniferous stems and photosynthetic, branched, vegetative stems ( Hauke, 1978 ). No such dimorphism occurs in the subgenus Hippochaete ( Hauke 1960). Although the chromosome number (n=108) is the same for all *Equisetum* species, the subgenus *Hippochaete* has larger chromosomes than those of subgenus *Equisetum* ( Hauke, 1978 ).

### 1.7. Therapeutical uses of *Equisetum* arvense

Multiple research studies have outlined various biological impacts of *Equisetum* arvense L. extract or tea containing natural extract, including antioxidant, anti-inflammatory, antibacterial, antifungal, vasorelaxant, neuroprotective, cardioprotective and antiproliferative effects( Makia, R *et al.*, 2022)

## MATERIALS AND METHODS

### 2.1 Plant Collection

The *Equisetum* plants were collected from their natural habitat in Tehsil Domail, District Bannu, Khyber Pakhtunkhwa, Pakistan, while *Syzygium cumini* was gathered from various localities within the same district.

### 2.2 Chemicals for Biological Activities

For the DPPH assay, 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma Germany. The dried methanolic extracts of *Equisetum* and *Syzygium cumini*, aluminum foil, and a double-beam spectrophotometer were utilized in the experiment (Khan *et al.*, 2023).

### 2.3 Preparation of Methanolic Extract of *Equisetum* and *Syzygium cumini*

The plants were collected in January 2024 from the District of Bannu, Pakistan. The samples were air-dried in a shaded area for 25 days. The dried plants were ground into a fine powder using a pestle and mortar. The powdered plant material was submerged in 80% methanol and kept at room temperature for 72 hours with frequent agitation. After 72 hours, the mixture was filtered through Whatman filter paper, and the filtrate was left at room temperature to dry. The gummy methanolic extract was lyophilized and stored in Falcon tubes for future use (Khan *et al.*, 2023).

### 2.4 Antioxidant Assay

The DPPH assay was conducted using a previously published protocol with slight modifications (Gyamfi *et al.*, 1999). In this assay, 100 µL of each plant extract at different concentrations (100 µg/mL, 500 µg/mL, and 1000 µg/mL) and an ascorbic acid solution were mixed with 900 µL of DPPH. The samples were incubated at 25°C in the dark for 30 minutes, and the absorbance was measured at 517 nm using a double-beam spectrophotometer. The percentage of DPPH radical scavenging activity was calculated using the following equation:

Percentage DPPH free radicals scavenging effect = (A1- A2/A1) × 100

Where A1= the absorbance of DPPH (control) and A2 = the absorbance in the presence of samples (Khan, S.*et al.*, 2023).

## RESULTS

### 3.1 Antioxidant Activity of *Equisetum*

The scavenging activity of *Equisetum* extract was tested at different concentrations: 100 µg/mL, 500 µg/mL, and 1000 µg/mL. The results showed an increase in antioxidant activity with higher extract concentrations, with 23%, 41%, and 88% scavenging activity observed at 100 µg/mL, 500 µg/mL, and 1000 µg/mL, respectively (Khan *et al.*, 2023).

**Table No 1. Comparing of Plant Extract *Equisetum* with stander Ascorbic Acid**

| A.A. 100µg/ml | P.E. 100µg/ml | A.A. 500µg/ml | P.E. 500µg/ml | A.A. 1000µg/ml | P.E. 1000µg/ml |
|---|---|---|---|---|---|
| 79% | 23% | 91% | 41% | 97% | 88% |

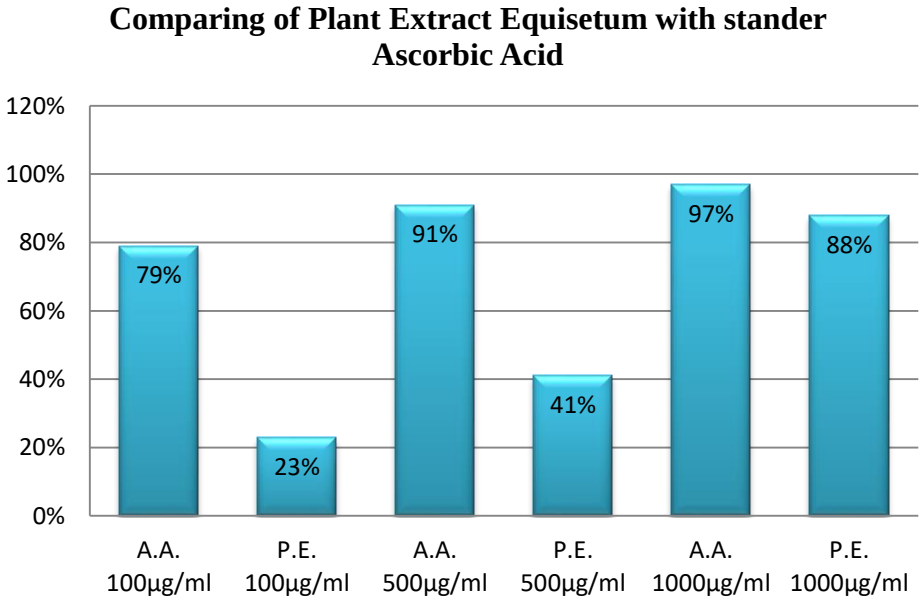



Figure 04: Comparison of *Equisetum* Extract with Standard Ascorbic Acid

### 3.2 Antioxidant Activity of *Syzygium cumini*

The antioxidant activity of *Syzygium cumini* extract also increased with rising concentrations. At 100 µg/mL, 500 µg/mL, and 1000 µg/mL concentrations, the scavenging activity was 36%, 59%, and 88%, respectively, as shown in Table 2 (Khan *et al.*, 2023).

**Table No 2. Comparing of *Syzygium cumini* with standard ascorbic acid**

| A.A. 100µg/ml | P.E. 100µg/ml | A.A. 500µg/ml | P.E. 500µg/ml | A.A. 1000µg/ml | P.E. 1000µg/ml |
|---|---|---|---|---|---|
| 79% | 36% | 91% | 59% | 97% | 88% |

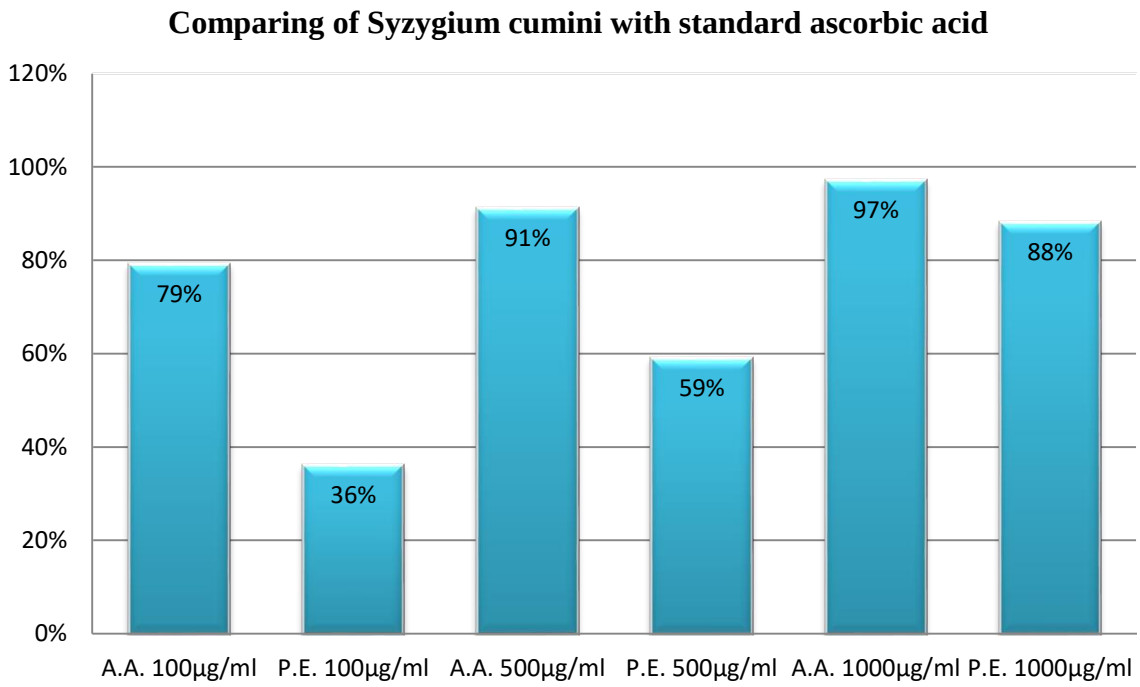



Figure 05: Comparing of *Syzygium cumini* with standard ascorbic acid

### 3.3 Combined Antioxidant Activity of *Equisetum* and *Syzygium cumini*

The combined extract of *Equisetum* and *Syzygium cumini* demonstrated a synergistic effect, enhancing the antioxidant activity. At concentrations of 100 µg/mL, 500 µg/mL, and 1000 µg/mL, the scavenging activities were 19%, 66%, and 92%, respectively (Khan *et al.*, 2023).

**Table No 3. Comparing of *Equisetum* and Sysygium cumini with standard ascorbic acid**

| A.A. 100µg/ml | P.E. 100µg/ml | A.A. 500µg/ml | P.E. 500µg/ml | A.A. 1000µg/ml | P.E. 1000µg/ml |
|---|---|---|---|---|---|
| 79% | 19% | 91% | 66% | 97% | 92% |

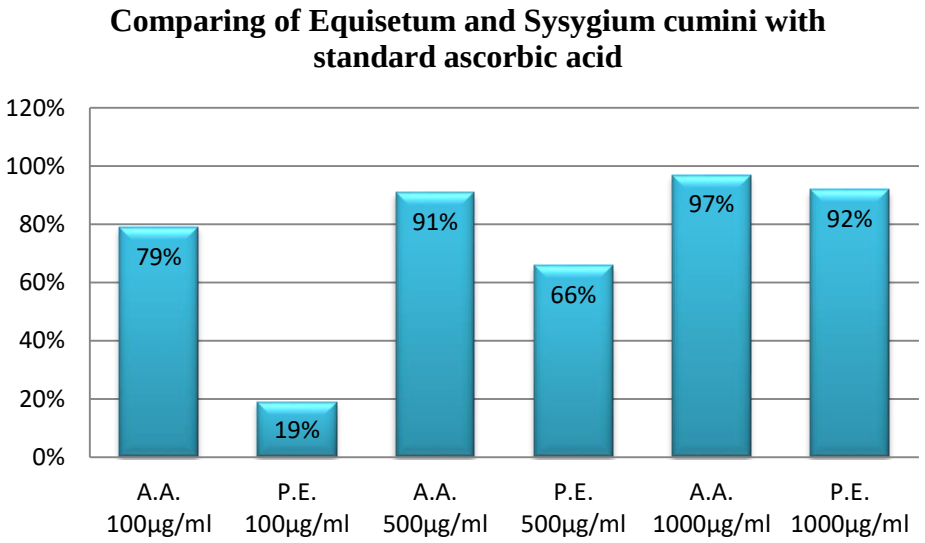



Figure 05: Comparison of Combined Extract with Standard Ascorbic Acid

## DISCUSSION

The antioxidant potential of *Equisetum* and *Syzygium cumini* methanolic extracts was evaluated using the DPPH assay. *Syzygium cumini* demonstrated higher antioxidant activity than *Equisetum*, with a significant increase in activity as the concentration of the extract increased. These results align with previous studies that have highlighted the medicinal properties of these plants due to their phytochemical composition, including tannins, saponins, and flavonoids (Rehman *et al.*, 2018). These compounds are known to exhibit antioxidant, antifungal, and antimicrobial properties, contributing to the overall medicinal value of the plants (Khan *et al.*, 2023).

The combined extract of *Equisetum* and *Syzygium cumini* showed an even greater antioxidant effect, suggesting a potential synergistic interaction between the two plant species. This enhanced activity highlights the potential of using plant combinations in developing natural antioxidants for food preservation and health-related applications (Gyamfi *et al.*, 1999).

## CONCLUSION AND FUTURE RECOMMENDATIONS

In conclusion, the methanolic extract of *Syzygium cumini* exhibited better antioxidant properties compared to *Equisetum*, while the combined extract of both plants demonstrated a synergistic increase in antioxidant activity. These findings suggest the potential application of these extracts as natural antioxidants in food preservation and other industries. Future research should focus on exploring their effectiveness in meat and animal products (Khan *et al.,* 2023).

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