ORIGINAL ARTICLE

Comparative Assessment of Synthetic and Natural Antioxidants: Synergistic Interactions Between Ebselen and *Stigma maydis* Extracts

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Abstract

Aim: To explore the hydro-ethanolic Stigma maydis extract antioxidant activity alone and in combination with Ebselen, a water-soluble vitamin E antioxidant, through several in vitro parameters. **Material and Methods:** After authentication, the extract was prepared by boiling and filtering 20g of corn silk. Various concentrations (20–100 µg) were tested using DPPH, ABTS, catalase, hydroxyl, and nitric oxide scavenging assays. Antioxidant activity was measured by absorbance changes at specific wavelengths, and results were expressed as scavenging percentages. Data were analyzed using mean and standard deviation. **Results and Discussion:** The strong antioxidant property was observed as 44%, 62%, 67%,75% and 88% of scavenging activity inhibition against DPPH, 36%, 54%, 71%, 76% and 84% of ABTS scavenging activity, 35%, 54%, 71%, 79% and 85% against catalase, 30%, 46%, 61%, 69% and 80% of hydroxyl radical scavenging activity and 25%, 45%, 61%, 73% and 82% of nitric oxide scavenging activity was noted when the hydro-ethanolic S. maydis extract combined with Ebselen. **Conclusion:** Hydro-ethanolic S. maydis extract combined with Ebselen showed strong antioxidant properties that may be used to treat various life-threatening diseases.

Key words: Antioxidant, ebselen, scavenging activity, *Stigma maydis*, synergism

INTRODUCTION

The antioxidant is an important molecule that inhibits or delays the process of oxidation by dismissing the oxidation chain reaction and protecting the cell damage from free radicals and these free radicals are short-lived molecules and they are reactive and unstable owing to its electron unpairness; hence, they intend to attach with other molecules to get stability.[1-4] Moreover, these molecules develop free radicals when they are attacked by external sources such as pollutants, ultraviolet radiation, drugs, smoke, which can lead to cell damage; and also during elevated free radicals' production and antioxidant production scarcity resulting in oxidative stress that is answerable for several life intimidating diseases including myocardial infarction, cancer, diabetes, and stroke.[5-9] In addition, the oxidative stress triggered by free radicals as well as their derivatives causes disturbances in redox homeostasis.[10]

However, the increasing oxidative stress situation triggers the cell defense system or undergoes apoptosis which affects many cellular processes such as core signaling pathways that are mainly related to the development of chronic or systemic disorders such as cancer and aging.[11] The antioxidants such as endogenous or exogenous are required for preventing this condition, but occasionally, the endogenous antioxidants including glutathione peroxidase, catalase, and superoxide dismutase, are not able to scavenge the free radical's overproduction.[12] In such cases, the exogenous antioxidants are tremendously needed to scavenge the free radicals by promoting the endogenous antioxidant function.[13] To improve

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the antioxidant process, the natural antioxidants are required for scavenging activity that is non-toxic, safe, and stable to prevent oxidative stress. Consequently, the plant (edible plants) derived molecules are effectively used for the prevention or treatment of various disorders. Therefore, several studies have reported the health benefits of dietary plants that contain biologically active components that have strong antioxidant activity.[14-19] Hence, numerous studies have reported on plant extracts for their free radical scavenging activity as well as lipid peroxidation inhibition.[20-22] Based on the evidence, the present study explored the antioxidant property of corn silk (*Stigma maydis*) alone and in combination with Ebselen, a synthetic antioxidant compound using different parameters.

MATERIALS AND METHODS

Plant authentication

The plant part used in the present investigation was identified as *S. maydis* by Dr. Shamna, MD, Chief Drug Analyst, Deseeya Ayurvedic Pharmacy, Calicut, India, with authentication number DAP/PI/31/2024.

Extract preparation

The corn silk (*S. maydis*) freshly collected was weighed (20 g) after washing with fresh water and added to an Erlenmeyer flask and the flask was heated for 20 min at boiling temperature and fluid was subjected to filtration using filter paper Whatman no.1 and the concentrated filtrate was used for all the studies.[23] For the study, to make a combination solution, corn silk extract 1 mg was mixed with 1 mg of ebselen and used for analysis, and also, for all the assays, 100 µg, 80 µg, 60 µg, 40 µg, and 20 µg from *S. maydis* extract and in combination with ebselen (100 µg, 80 µg, 60 µg, 40 µg, and 20 µg) were used.

2, 2-diphenyl-1-picryhydrazyl (DPPH) radical scavenging assay

To check the antioxidant property of hydroethanolic *S. maydis* extract alone and in combination with ebselen, the DPPH assay was used as indicated before.[24] Shortly, the hydroethanolic *S. maydis* extract alone (100 µg, 80 µg, 60 µg, 40 µg, and 20 µg) and in combination with ebselen (100 µg, 80 µg, 60 µg, 40 µg, and 20 µg) were added to test tubes and made to 3 mL and the methanolic DPPH (0.1 mM) was added and these mixtures were kept for 30 min in the dark condition. Then, the incubated solution was monitored for pink color formation which was read at 517 nm to calculate the extract alone and in combination radical scavenging activity percentage after treatment.

Scavenging activity = 100× (blank OD−sample OD)/blank OD

(2,2'-azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) (ABTS) scavenging assay

The ability of hydroethanolic *S. maydis* extract alone and in combination with ebselen to scavenge the ABTS was studied as mentioned earlier.[25] To start the experiment, the solution of ABTS was prepared in water by mixing 2.45 mM potassium persulfate ($K_2S_2O_8$) and 7 mM ABTS following an incubation at room temperature under dark conditions for 16 h. Then, the prepared mixture was diluted using 0.1 M sodium phosphate buffer (pH 7.4) to attain an absorbance value of 0.750 ± 0.025 at 734 nm and the adjusted solution was used for the assay. The concentrations (100 µg, 80 µg, 60 µg, 40 µg, and 20 µg) from *S. maydis* extract and in combination with ebselen (100 µg, 80 µg, 60 µg, 40 µg, and 20 µg) were added to a test containing 1 mL of $ABTS^+$ solution which was allowed for 6 min and measured at 734 nm to calculate the scavenging percentage of extract alone and in combination with ebselen.

ABTS scavenging activity = 100 × (control OD−sample OD)/control OD

Catalase assay

The catalase activity of hydroethanolic *S. maydis* extract alone and in combination with Ebselen was investigated through catalase assay as indicated previously.[26] Briefly, the varying concentrations ranging from 20 µg to 100 µg of *S. maydis* extract and in combination with ebselen (20 µg–100 µg) were added to the phosphate buffer (1.5 mL), followed by 60 mM hydrogen peroxide addition. The absorbance measured at 240 nm indicate the direct proportion to H_2O_2 decomposition which was used for calculating the percentage of radical scavenging activity of extract alone and in combination with Ebselen.

Scavenging activity = 100 × (control OD−sample OD)/control OD

Hydroxyl radical scavenging activity

To ability of hydroethanolic *S. maydis* extract alone and in combination with ebselen on hydroxyl radical scavenging activity was examined as indicated before.[27] In short, $FeCl_3$ (1 mM), 1,10-phenanthroline (1 mM), phosphate buffer (0.2 M; pH 7.8), and H_2O_2 (0.17 M) were added to a test tube which contain different ranges of *S. maydis* extract concentrations (20 µg–100 µg) alone and in combination with ebselen (20 µg–100 µg) and allowed for 5 min to react at room temperature followed by final product measurement at 560 nm. The hydroxyl radical scavenging activity after treatment was found out by the end product.

Scavenging activity = 100 × (control OD−sample OD)/control OD

Nitric oxide scavenging activity

The capacity to scavenge nitric oxide after treatment with the extract alone and in combination with ebselen was investigated as illustrated before.[28] In brief, the reaction mixture was made using sodium nitroprusside (10 mM) in phosphate buffer (0.5 M; pH 7.4) and the different ranges of *S. maydis* extract concentrations (20 µg–100 µg) alone and in combination with ebselen (20 µg–100 µg) and incubated at 37°C for 1 h. Then, to the mixture, the gross solution was added equally which read at 540 nm to calculate radical scavenging activity percentage after treatment with extract alone and in combination with ebselen.

Scavenging activity = 100 × (control OD−sample OD)/ control OD

Statistical analysis

The mean and standard deviation were calculated to make the standard error for all the experiment.

RESULTS

DPPH radical scavenging assay

Antioxidant property of hydroethanolic *S. maydis* extract alone and in combination with Ebselen was checked and the calculated scavenging activity is reported in Figure 1. The figure demonstrated the antioxidant property of the extract alone and in combinations with Ebselen, and varying concentrations of *S. maydis* extract alone caused 35%, 51%, 59%, 67%, and 74% scavenging activity. Whereas, when the *S. maydis* extract was combined with ebselen, it showed 44%, 62%, 67%, 75%, and 88% of scavenging activity which clearly demonstrated that the combination of corm silk and ebselen had increased antioxidant activity.

ABTS scavenging assay

The ability to scavenge the ABTS was studied after treatment with hydroethanolic *S. maydis* extract alone and in combination with ebselen, and the quantified scavenging activity is presented in Figure 2. As observed in the figure, the hydroethanolic *S. maydis* extract alone exhibited 29%, 48%, 52%, 55%, and 65% of scavenging activity and the extract combined with ebselen showed 36%, 54%, 71%, 76%, and 84% of ABTS scavenging activity.

Catalase assay

The hydroethanolic *S. maydis* extract alone and in combination with ebselen catalase activity was investigated, and the quantified scavenging activity is mentioned in Figure 3 The various concentrations of hydroethanolic *S. maydis* extract revealed the 21%, 42%, 56%, 70%, and 76% catalase scavenging activity, and an increasing scavenging activity as 35%, 54%, 71%, 79%, and 85% was noted when the extract combined with ebselen.

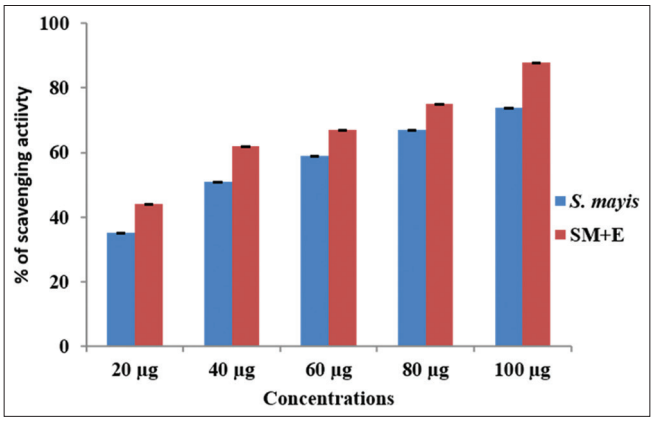

Figure 1: Antioxidant property of hydroethanolic *Stigma maydis* extract alone and in combination with ebselen was quantified and the calculated scavenging activity was reported as graph

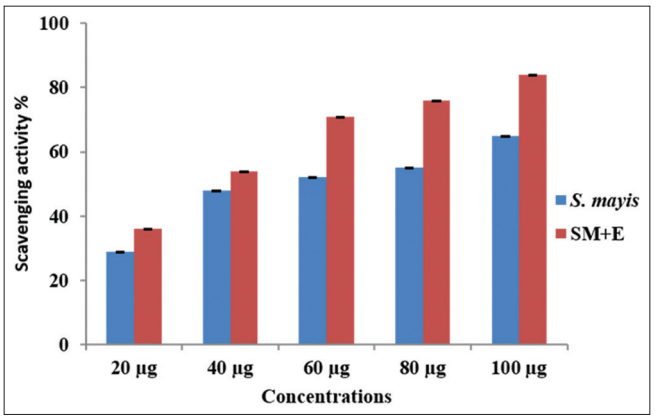

Figure 2: Quantification of (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) scavenging activity of hydroethanolic *Stigma maydis* extract alone and in combination with ebselen

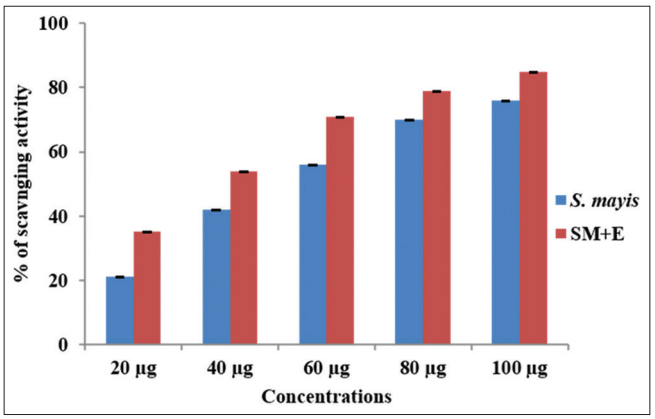

Figure 3: Quantification of catalase scavenging activity of hydroethanolic *Stigma maydis* extract alone and in combination with Ebselen

Hydroxyl radical scavenging activity

The hydroethanolic *S. maydis* extract alone and in combination with ebselen ability to scavenge the hydroxyl radical after treatment was investigated, and the quantified scavenging percentage is presented in Figure 4. The varying hydroethanolic *S. maydis* extract concentrations showed 23%, 32%, 43%, 56%, and 70% of hydroxyl radical scavenging activity after treatment, and also, the extract showed 30%, 46%, 61%, 69%, and 80% of hydroxyl radical scavenging activity when it was combined with Ebselen.

Nitric oxide scavenging activity

The hydroethanolic *S. maydis* extract alone and in combination with ebselen ability for nitric oxide scavenging activity was explored after treatment and the quantified scavenging activity is displayed in Figure 5. The different concentrations of *S. maydis* extract showed 21%, 40%, 48%, 60%, and 69% of nitric oxide scavenging activity, and also, nitric oxide scavenging activity was increased as 25%, 45%, 61%, 73%, and 82% when the hydroethanolic *S. maydis* extract combined with ebselen.

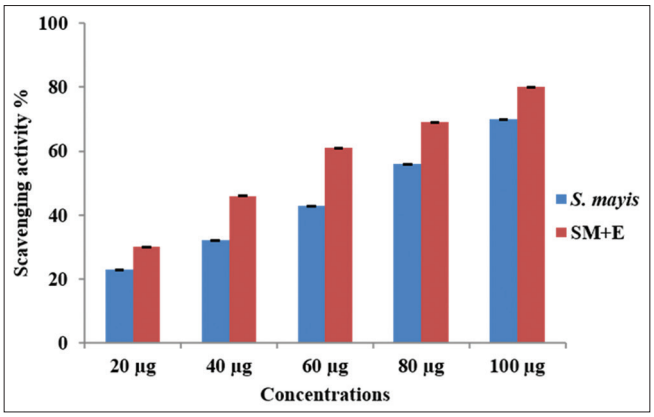

Figure 4: Quantification of hydroethanolic *Stigma maydis* extract alone and in combination with Ebselen on hydroxyl radical scavenging activity after treatment

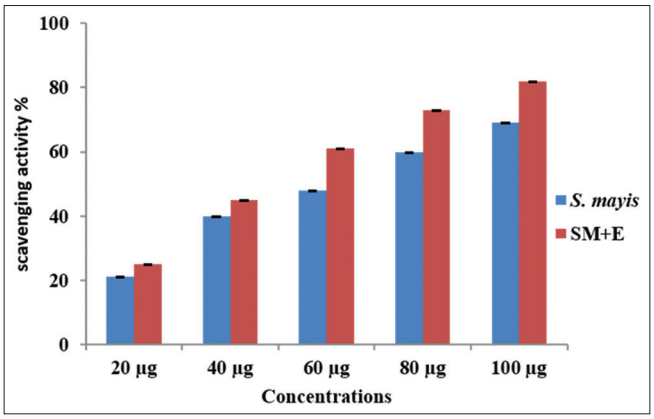

Figure 5: Nitric oxide scavenging activity of hydroethanolic *Stigma maydis* extract alone and in combination with ebselen

DISCUSSION

The molecules possessing antioxidant properties are used for preventing or delaying the oxidative process which are responsible for a variety of life-threatening diseases such as cardiovascular diseases, diabetes, and cancers.[29] Usually, the body has inert antioxidants to prevent the oxidation process and it is enhanced during additional or external antioxidants such as synthetic or dietary plant-derived compounds. Hence, this study explored the hydroethanolic *S. maydis* extract alone and in combination with ebselen antioxidant property through various *in vitro* parameters including ABTS, DPPH, catalase, hydroxy radical and nitric oxide scavenging activity and found a promising antioxidant in alone and their combination. In support of this, a well-known plant material *S. maydis* was investigated their antioxidant property and the silk corn chemical compositions were identified. The antioxidant properties were explored by ABTS, ferric ion reducing antioxidant potential, and DPPH assays, resulting in potent scavenging activities than Trolox, a water-soluble vitamin E with strong antioxidant activity. The *S. maydis* antioxidant property was mediated through an important phytochemical flavonoid.[30] Another study reported that the phytochemicals of *S. maydis* that is responsible for the antioxidant property of various stages of *S. maydis* include steroids, flavonoids, polysaccharides, and also, polyphenols. Moreover, the *S. maydis* antioxidant property was achieved by electron paramagnetic resonance technique, DPPH, ABTS free radical measurement, copper ion reductive capacity, and ferric ion-reducing antioxidant power and found that antioxidant property was associated with stages involved in corn silk maturation, and also, the higher antioxidant was attained in silk mature stage, the silky stage followed by the milky stage, respectively, which suggest that mature silk corn has more potent antioxidant property.[31] Similarly, the corn silk was extracted using solid-to-solvent ratio of 1:17.5, and acetone and isolated compounds (E)-4-(4-hydroxy-3-methoxyphenyl) but-3-en-2-one (2) and friedelin exhibited promising antioxidant through DPPH, H_2O_2, and ABTS % inhibition which suggested that *S. maydis* may be used as low-cost natural antioxidant.[32] Interestingly, the various solvents such as ethanol, petroleum, ethyl acetate, water, and n-butanol were used to identify the phytochemicals including phenols and flavonoids which have antioxidant activity by showing increased total antioxidant activity, strong scavenging activity against hydroxyl radicals and DPPH as well as high reducing power and also, had the anti-diabetic which suggested that, corn silk can contribute for therapeutic benefits as antioxidant that can be developed for treating many life threatening diseases.[33,34]

CONCLUSION

The study highlighted the antioxidant property of hydroethanolic *S. maydis* extract alone and in combination with ebselen through DPPH, ABTS, catalase, H_2O_2, and

nitric acid assays which explored the scavenging activity inhibition. Moreover, the hydroethanolic *S. maydis* extract alone exhibited promising antioxidant activity, and the strong antioxidant activity was observed when combined with a water-soluble antioxidant agent, ebselen. Overall, the corn silk had strong antioxidant when combined with ebselen and this combination can be used as natural antioxidant for treating many life-threatening diseases.

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