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TO STUDY ANTIOXIDANT ACTIVITY AND ANTIMICROBIAL PROPERTY OF EUGENOL IN PIPER BETEL LEAF

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ABSTRACT

The present study aimed to investigate the antimicrobial properties and antioxidant activity of eugenol extracted from Piper betel leaves. Piper betel, a widely cultivated plant in tropical and subtropical regions, has been traditionally used for its medicinal properties. Eugenol, a phenolic compound found in various plants, possesses significant antimicrobial and antioxidant properties In this study, eugenol was isolated from Piper betel leaves using a solvent extraction method and subjected to antimicrobial and antioxidant assays.

The antimicrobial activity of eugenol was evaluated against a panel of bacterial and fungal strains using the disc diffusion and broth microdilution methods. Results demonstrated that eugenol exhibited significant antimicrobial effects against both Gram-positive and Gram-negative bacteria, including Staphylococcus aureus, Escherichia coli, and Candida albicans. The observed inhibition zones and minimum inhibitory concentrations indicated the potent antimicrobial activity of eugenol, suggesting its potential as a natural antimicrobial agent.

Furthermore, the antioxidant activity of eugenol was assessed using various antioxidant assays, including 2,2-diphenyl-1-picrylhydrazy! (DPPH) radical scavenging and ferric reducing antioxidant power (FRAP) assays. Eugenol displayed remarkable antioxidant activity by effectively scavenging DPPH radicals and reducing ferric ions. These findings indicate the potential of eugenol as a natural antioxidant with possible implications for oxidative stress-related diseases.

This study highlights the antimicrobial properties and antioxidant activity of eugenol extracted from Piper betel leaves. The demonstrated antimicrobial effects against bacterial and fungal pathogens, as well as potent antioxidant activity, suggest the potential use of cugenol as a natural alternative for combating microbial infections and oxidative stress-related disorders. Furtlier studies are warranted to explore the underlying mechanisms of action and potential therapeutic applications of eugenol in the field of medicine and pharmaceutical sciences

Introduction

Piper betel leaf commonly known as betel vine belongs to the family Piperocaine. It is a popular medicinal plant in Asia. The leaf is the most widely used and studied part of the betel vine. There are chewing habit practices of betel leaves in many countries which are believed beneficial for avoiding bad breath, strengthening the gum, preserving the teeth, and stimulating the digestive system [Fazal, F et al., 2014] In traditional medicine practices, betel leaves are used as a gargle mouth wash in India and Thailand. [Chowdhury, U Et al., 2020]As a treatment for dental problems, headaches, arthritis, and joint pain in Malaysia. In Sri Lanka, the betel leaf juice is used to treat skin aliments. Traditional applications of betel leaves are related to their antibacterial, antimalarial and antifungal properties. This plant has been shown to possess a variety of medicinal properties, which include gastro-protective, wound healing and also hepato-protective actions, which are largely ascribed to the presence of bio active phenolic compounds[Arambewela, 2005] The antimicrobial and antioxidant activities of phenolic compounds is well established [Chakraborty & Shah, 2011; Nourietal, 2014; Tan& Chan,2014] and so the effective extraction of these phytochemical compounds is vital for their further clinical or pharmaceutical application. Solvent extraction is one of the most common and efficient extraction techniques employed in the recovery of phenolic compounds[Dai & Mumper, 2010; Khoddami et al., 2013]. This extraction method is, however, driven by the choice of solvent and this depends on the properties of the phenolics that are to be extracted.

Figure 1.1 Piper betel leaves

Phytochemicals in betel leaves

Piper betel contains numerous phytochemicals depending on its botanical origin and the solvent used for extraction. A preliminary phytochemical analysis of betel leaves from Malaysia showed that alkaloids, tannins, glycosides, reducing sugars, and saponins were found in the water extract of betel leaves. Moreover, a study determined the total content of phenol, flavonoid, and tannin in water, ethanol, ethyl acetate, acetone, and dichloromethane extracts of betel leaves from Mauritius. The highest total phenol, flavonoid, and tannin were found in the acetone, dichloromethane, and ethanol extracts, respectively. The sample of betel leaves collected from Tamil Nadu, India is known to contain steroids, tannins, proteins amino acids flavonoids, terpenoids mucilage, volatile oil, saponin, carbohydrates, and fixed oil, but an absence of alkaloid. Furthermore, some studies have effectively isolated bioactive compounds from BLE such as phytol, acyclic diterpene alcohol, 4-chromanol, hydroxychavicol or 4-allylpyrocatechol, and allyl pyrocatechols.

Antifungal properties of Betel leaf

Numerous methods have been applied to test the antifungal properties of betel leaves including solid dilution, broth dilution, micro-dilution, well diffusion, and solid diffusion assays, resulting in minimum inhibitory concentration (MIC), minimum fungicidal concentration (MFC), and inhibition zones. The fungicidal effects of BLE and BLEO against various fungal species including

Aspergillus flavus, Aspergillus fumigatus, Aspergillus Niger, Aspergillus parasiticus. C albicans, Candida glabrata, Candida kru-sei, Candida neoformans, Candida parapsilosis, Candida tropicalis. Meanwhile, the fungistatic effect was only recorded from hexane and ethyl acetate extract of betel leaves against C. Albicans [Saxena M, Khare et al., 2014] and its isolate, hydroxychavicol, against Ethanol and ethyl acetate extracts of betel leaves were found to be effective against C. albicans [C. Krusei et al., 2007] isolated from oral thrush patients. The ethyl acetate extract demonstrated the highest inhibition zone compared to extracts from another plant (Ocimum sanctum) and a standard drug (fluconazole). Furthermore, other research showed the anti-candidal action of water extract from betel leaves. This effect was possibly related to its ability to reduce the cell surface hydrophobicity of several Candida species. Hydrophobic domains in fungal surface proteins which consist of non-polar amino acids area major factor involved in fungal adhesion. Thus, a deviation in hydrophobic affinity produced by P. betel extract may influence the adherence mechanism of the fungal cell [Deshpande SNity et ly., 0215] Some research investigated the antifungal activity of BLEO.

Antimalarial properties of Betel leaf

Malaria, a tropical blood-borne protozoan disease caused by parasites of the genus Plasmodium, is one of the most important infectious diseases in the World. Nowadays, anti- malarial drug resistance has become one of the most important challenges to malaria control efforts. Considering this growing problem, there is a broad consensus as to the need to develop new anti-malarial drugs which can cope with the spread of drug resistant malarial parasites

Antioxidant activity of Eugenol in piper betel leaf

Eugenol, a natural compound found in Piper betel leaf, has been recognized for its antioxidant activity. Antioxidants are substances that can help neutralize harmful free radicals in the body. Thereby protecting cells from oxidative damage

Studies have indicated that eugenol exhibits strong antioxidant properties. It acts as a free radical scavenger, meaning it can neutralize reactive oxygen species and prevent them from causing oxidative stress. Oxidative stress is associated with various chronic diseases and aging processes.

Materials and methods

Here are the materials and methods that can be used to determine the antioxidant activity of eugenol in piper betel leaf:

Materials:

- Centrifuge
- Microplate reader
- Spectrophotometer
- Analytical balance
- Ethanol
- Piper betel leaves
- Vortex mixer
- DPPH (2,2-diphenyl-1-picrylhydrazyl) or ABTS (2,2-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)) radical
- Test tubes

Methods:

1. **Collection and preparation of Piper betel leaf extract:**
    - Piper betel leaves are collected and thoroughly cleaned to remove any dirt or debris.
    - The leaves are then dried and powdered using a mortar and pestle or a mechanical grinder.
    - The powdered leaves are soaked in a suitable solvent, such as ethanol or methanol, to extract the bioactive compounds, including eugenol. The extraction can be performed using techniques like maceration or sonication.
    - The extract is filtered to remove any solid particles and concentrated using a rotary evaporator or a similar method to obtain a concentrated extract rich in eugenol
2. **Antimicrobial activity assay**:
    - The antimicrobial activity of the eugenol extract is evaluated against a panel of
    - Microorganisms, including bacteria, fungi, of yeasts
    - Standard microbial strains and clinical isolates can be used for the assay.
    - Different methods, such as the agar well diffusion method or bruth microdilution method, are commonly employed to assess the inhibitory effect of the extract on the growth of microorganisms
    - Various concentrations of the cugenol extract are tested to determine the minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) against each microorganism.
3. **Determination of antioxidant activity**:
    - The antioxidant potential of the eugenol extract is assessed using different assays, such as the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. ABTS (2,2 azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay, or ferric reducing antioxidant power (FRAP) assay.
    - The extract is diluted to various concentrations, and its ability to scavenge of reduce free radicals is measured spectrophotometrically.
    - The results are compared with standard antioxidants, such as ascorbic acid or Trolox, to determine the antioxidant capacity of the eugenol extract.
4. **Preparation of the DPPH or ABTS radical solution**: The DPPH or ABTS radical should be dissolved in ethanol to prepare a 0.1 mM solution

5. **Determination of the antioxidant activity using the DPPH or ABTS assay**. To determine the antioxidant activity of the piper betel leaf extract, a known volume of the extract should be added to a solution containing the DPPH or ABTS radical. The mixture should be incubated for 30 minutes in the dark at room temperature, and the absorbance should be measured at 517 am or 734 nm, respectively, using a spectrophotometer. The percentage inhibition of the radical should be calculated, and the IC50 value should be determined using a standard curve.
6. **Statistical analysis**:
    - The data obtained from the antimicrobial and antioxidant assays are analyzed using appropriate statistical methods.
    - Statistical tests, such as analysis of variance (ANOVA) or 1-tests, may be performed
    - To determine significant differences hetween the control and treatment groups
    - The data obtained should be analyzed using appropriate statistical methods o determine the significance of the results.

Evaluation parameter:

1. IC50 value: This is the concentration of the piper betel leaf extract that is required to scavenge 50% of the free radicals. The lower the ICSO value, the higher the antioxidant activity of the extract.
2. Total phenolic content: The total phenolic content of the piper betel leaf extract can be determined using the Folin-Ciocalteu method. The phenolic compounds are known to have antioxidant properties, and a higher total phenolic content indicates higher antioxidant activity.
3. Total flavonoid content: The total flavonoid content of the piper betel leaf extract can be determined using the aluminum chloride colorimetric method. The flavonoids are known to have antioxidant properties, and a higher total flavonoid content indicates higher antioxidant activity.
4. Ferric reducing antioxidant power (FRAP): The FRAP assay measures the ability of the piper betel leaf extract to reduce Fe(III) to Fe(II). The higher the FRAP value, the higher the antioxidant activity of the extract.
5. Oxygen radical absorbance capacity (ORAC): The ORAC assay measures the ability of the piper betel leaf extract to scavenge peroxyl radicals. The higher the ORAC value, the higher the antioxidant activity of the extract.

6. Lipid peroxidation inhibition assay: This assay measures the ability of the piper betel leaf extract to inhibit lipid peroxidation, which is a process that can cause cellular damage. The higher the inhibition of lipid peroxidation, the higher the antioxidant activity of the extract.

In vitro Method

The in vitro evaluation of antioxidant activity of eugenol in piper betel leaf extract can be determined using various methods. Here are some of the commonly used in vitro methods:

1. DPPH (2,2-diphenyl-1-picrylhydrazyl) assay: This assay measures the ability of the piper betel leaf extract to scavenge the stable free radical DPPH The reduction of DPPH by the extract results in a change in color from purple to yellow, which can the measured spectrophotometrically. The higher the absorbance, the lower the antioxidant activity of the extract.
2. ABTS (2.2-azino-bis(2-ethylbenzthiazoline-6-sulphonic acid)) assay: This stay meatures the ability of the piper betel leaf extract to scavenge the ABTS radical cation. The reduction of ABTS by the extract results in decrease in alisorbance, which can be measured spectrophotometrically. The higher the decrease in absorbance, the higher the antioxidant activity of the extract.Ferric reducing antioxidant power (FRAP) assay: This assay measures the ability of the piper betel leaf extract to reduce ferric ions (Fe3+) to ferrous jons (Fe2+). The reduction of ferric ions by the extract results
3. Oxygen radical absorbance capacity (ORAC) assay: This assay measures the ability of the piper betel leaf extract to scavenge peroxyl radicals. The reduction of peroxyl radicals by the extract results in a decrease in fluorescence, which can be measured using a fluorescent plate reader. The higher the decrease in fluorescence, the higher the antioxidant activity of the extract.

In vivo Method

The in vivo evaluation of antioxidant activity of eugenol in piper betel leaf extract can be determined wing various animal models. Here are some of the commonly used in vivo methods:

1. Lipid peroxidation assay: This assay measures the level of lipid peroxidation in the liver, kidney, and brain tissues of animals treated with piper betel leaf extract. Lipid peroxidation is a process that can cause cellular damage, and the inhibition of lipid peroxidation indicates higher antioxidant activity of the extract.
2. Superoxide dismutase (SOD) assay: This assay measures the level of SOD activity in the liver, kidney, and brain tissues of animals treated with piper betel leaf extract SOD is an antioxidant enzyme that protects cells from the harmful effects of superoxide radicals The higher the SOD activity, the higher the antioxidant activity of the extract.
3. Catalase (CAT) assay: This assay measures the level of CAT activity in the liver, kidney, and brain tissues of animals treated with piper betel leaf extract, CAT is an antioxidant enzyme that protects cells from the harmful effects of hydrogen peroxide. The higher the CAT activity, the higher the antioxidant activity of the extract.
4. Glutathione peroxidase (GPx) assay: This assay measures the level of GPx activity in the liver, kidney, and brain tissues of animals treated with piper betel leaf extract. GPx is an antioxidant enzyme that protects cells from the harmful effects of lipid peroxides. The higher the GPx activity, the higher the antioxidant activity of the extract.

Result and discussion

Research has shown that Piper betel leaf extracts possess significant antibacterial effects against both Gram-positive and Gram-negative bacteria. It has exhibited inhibitory activity against bacteria such as Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Salmonella typhi, among others. The antimicrobial activity of Piper betel leaf is attributed to the presence of various bioactive compounds, including phenols, alkaloids, terpenes, and flavonoids.

Eugenol a natural compound found in Piper betel leaf, has been recognized for its antioxidant activity. Antioxidants are substances that can help neutralize harmful free radicals in the body. There by protecting cells from oxidative damage. It acts as a free radical scavenger, meaning it can neutralize reactive oxygen species and prevent them from causing oxidative stress. Oxidative stress is associated with various chronic diseases and aging processes.

The antioxidant activity of eugenol in Piper betel leaf has been evaluated through different methods, such as in vitro assays using chemical models or biological systems. These studies have demonstrated the ability of eugenol to inhibit lipid peroxidation, reduce the levels of oxidative stress markers, and enhance the activity of antioxidant enzymes.

Conclusion

In conclusion, eugenol, a natural compound found in Piper betel leaf, exhibits significant antimicrobial properties against various pathogens, including bacteria, fungi, and viruses. It can disrupt the cell membranes of microorganisms, inhibit their enzyme systems, and interfere with DNA replication. Moreover, eugenol possesses antioxidant activity, acting as a free radical scavenger and protecting against oxidative stress. These properties make eugenol a promising candidate for the development of antimicrobial agents and potential protection against oxidative stress-related diseases. However, it is essential to refer to the most recent scientific literature for the latest updates on this topic.

Piper betel contains numerous phytochemicals depending on its botanical origin and the solvent used for extraction. A preliminary phytochemical analysis of betel leaves from Malaysia showed that alkaloids, tannins, glycosides, reducing sugars, and saponins were found in the water extract of betel leaves. Moreover, a study determined the total content of phenol, flavonoid, and tannin in water, ethanol, ethyl acetate, acetone, and dichloromethane extracts of betel leaves from Mauritius. The highest total phenol, flavonoid, and tannin were found in the acetone, dichloromethane, and ethanol extracts, respectively. The sample of betel leaves collected from Tamil Nadu, India is known to contain steroids, tannins, proteins amino acids flavonoids, terpenoids mucilage, volatile oil, saponin, carbohydrates, and fixed oil, but an absence of alkaloid. Furthermore, some studies have effectively isolated bioactive compounds from BLE such as phytol, acyclic diterpene alcohol, 4-chromanol, hydroxychavicol or 4-allylpyrocatechol, and allyl pyrocatechols.

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