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# A Literature Review of Antioxidant Activity of P. Betle Leaf Extract.

***Varsha S Lohar* , Pooja S Masha , Pratham R Mane , Aradhna B Mante , Dr.Ashish S Jain.***

**Affiliation :** Shri D.D Vispute College of pharmacy and research centre, New Panvel -410206
**Institutional address:** Shri D.D Vispute College of pharmacy and research centre, New Panvel -410206
**E-mail address:** loharvarsha344@gmail.com

**ABSTRACT:**

Piper betle leaf has been considered a natural antioxidant. The P. betle extract shows high antioxidant activity, mostly due to presence of phenolic and flavonoid compounds in the P. betle. Eugenol was discovered to possess antioxidant properties and it's percentage ie. (4.86%) was calculated from betel leaf using the Ultrasound extraction process and acidified water used as solvent. The P. betle leaf phenolic content were found to protect photosensitization- mediated lipid peroxidation in rat liver or by free radical scavenging activity and thus enhance antioxidant activity. In a few studies it also been reported that the P. betle with high antioxidant activities suppress the proliferation of cancer cells. In previous study, the study evaluated the anti-oxidative activity of the extract of P. betle leaves (PBLS) on the basis of Cu(2+)- mediated oxidation and it thus has ability to prevent foam cell formation in humans for oxidised low density lipoprotein (ox LDL)-induced lipid accumulation in macrophages. P. betle leaf have numerous properties like antiseptic, anti-oxidant, anti-cancer, anti-inflammatory. P. betle leaf contains many Phytochemicals such as alkaloids, flavonoids, tannis, essential oil, steroids, saponins and suger too.

**Keywords**: P.betle leaf; Anti-oxidant ; Eugenol ; Ultrasound extraction; Free radical.

## Introduction:

In human, the oxidation process during normal metabolism produces unstable molecule called free radicals. A molecule with one or more than one unpaired electron in its outer shell is known as free radicals. Free radicals causes damage to cell membrane and structure.An anti-oxidant is any substance that can delay or prevent the oxidation of a substrate and protect cells from the damage.[1]Anti-oxidant are the potential solution to the disease caused by free radical such as neurological disease, cancer, pulmonary disease and condiovascular diseases.[2] These free radicals can involve in the aging process, thus betel leaf extract can be used as anti -aging agent Anti-oxidant is used in inhibition of reactive oxygen species (ROS) for treating diseases.[ 3]

Mechanism of action:

The anti-oxidant substance works by scavenging activity on free radicals ie. Reactive oxygen species (ROS). From previous studies, it was reported that betel leaf extract exhibited the free radical scavenging activity.[4,5] In previous study, the study evaluated the anti-oxidate activity of the extract of Piper betle leaves (PBLs) on the basis of Cu(2+)-mediated oxidation and it thus has ability to prevent foam cell formation in human for oxidised low density lipoprotein (oxLDL)-induced lipid accumulation in macrophages. PBLs up regulated the protein levels of the class A and Class B scavenger receptors, its upstream regulator liver X receptor (LXR) and the membrane lipid transporter ABCA1 in macrophages that are exposed to oxLDL. This result minimise the damage of vessels caused by the oxLDL and to prevent foam cell formation and lipid accumulation.[6]

## Information about Piper betle leaf :

[34]

Piper betle L ; synonym: Piper betel Blanco or betel vine, belong to the family *Piperaceae*.[7] The betel leaf grows on a vine that makes roots. In India, betel leaves are heart-shaped and dark green. They are also referred to as Paan.[8] Leaf extract and Purified bioactive compounds of P. betle have many beneficial properties like antiseptic, anti-oxidant, anti-cancer, anti-inflammatory. P. betle contains many phytochemicals such as alkaloids, flavonoids, tannins, essential oil, steroids, saponins and sugar too.[9]India developed a broad variety of P. betle leaves that are traditionally and currently used as a mouth fresher after every meal in India. P. betle is also found and been used by various other countries like Malaysia, Thailand and Srilanka.[10] P. betle has a qualities including a potent pungent and aromatic flavor, so the Asian people utilze it as a masticator.[11]

**Chemical constituents:**

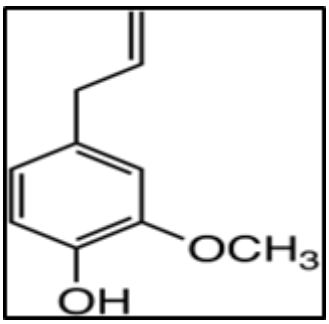
The main phyto-constituent of the betel leaf are mainly phenols & terpenoids. The preliminary studies of aqueous & methanol extract of betel leaves shows the presence of alkaloids, flavonoids, tannins, sterols, phenols, glycosides, saponins & terpenoids as chemical constituent.[7]

**Table no.1[8]**

| **Sr.no** | **Chemical compounds** | **Qt %** |
|---|---|---|
| 1 | Eugenol | 48.41%. |
| 2 | Acetyl Eugenol | 14.05%. |
| 3 | Alliylpyrocatechol diacetate | 0.71% |
| 4 | Allylpyrocatchol monoacetate | 0.23% |
| 5 | Camphene | 0.48% |
| 6 | Cyrophyllene | 1.3% |
| 7 | Chavibetol | 53% |
| 8 | Chavibetol acetate | 15.5% |
| 9 | Chavicol | 11.08% |
| 10 | Eucalyptol | 1.58% |
| 11 | Eugenol acetate | 28% |
| 12 | Methyl acetate | 1.45% |
| 13 | α-Copaene | 6.43% |
| 14 | α-Pinene | 0.23% |
| 15 | β-Cubebene | 13.06% |
| 16 | 1,8 cineol | 0.04% |
| 17 | 3, methoxy cinnamonaldehyde | 1.38% |

Eugenol is a phenolic compound from the class of phenylpropanoid. The chemical composition of Eugenol is (4-allyl 2-methoxyphenol). Eugenol is a natural antioxidant.[35]

Eugenol




Eugenol is pale yellow liquid and has an oily consistency. It has a spicy aroma . Eugenol is sparingly soluble in water and well soluble in organic solvent. Eugenol is a monoamine oxidase (MAO) inhibitor & it also exhibit micro- protective properties. It inhibits the generation of reactive oxygen species and prevents the formation of reactive forms of nitrogen & it also increases the cyto-antioxidant potential. The antioxidative property of Eugenol is due to its structure, it allows it to fix phenoxy radicals by receiving donated Hydrogen atoms.[36]

***Species:***

There are over 100 different types of species of betel leaf are discovered. Over 40 are found in India Only. From them, Bagerhati, Ghanagete & Kauri are considered to be rich in antioxidant.[14] There are mainly 5 cultivator of betel vine These care Desawari, Bangla, Kapoorin, Meetha & Sanchi. Kaptori & Sanchi are common in peninsular India. Bangla & Desivan are common in North India. Meetha is grown in West Bengali Only. The season for optimum cultivation are in between November to February.[13]

***Methods for extraction of Eugenol :***

1.Steam distillation method :-

-It is the most popular Method for isolating eugenol. In this procedure, essential oil is first collected from the plant.

-Eugenol is then extracted by mixing the essential oil with 3% solution of sodium potassium hydroxide. The product of this reaction is phenolic alkali salt.

- Next, the extracts insoluble component is using steam distillation or solvent extraction.[37]

2.Solvent extraction method :-

-Another Method for extracting ethanol is by employing different solvents, such as methanol, ethanol, and petroleum ether. Using an appropriate organic solvent in a Soxhlet system, extraction is performed.[15]

- The extracted materials are concentrated at 50°C to complete the process.

3.Ultrasound extraction method :-

-Ultrasonic baths and probes, which use piezoelectric transducers as their source of ultrasonic power, are typically used for UAE procedures. The solid matrix is distributed in the solvent in a stainless steel tank that is coupled to a transducer in an ultrasonic bath. Because of the probe's higher ultrasonic intensity (tip), probe-based systems are frequently chosen over bath systems and are effective tools for extracting bioactive chemicals due to their non-uniform.[16]Through the probe, the ultrasonic energy is emitted into the sample.[17]

-The diameter of the laboratory scale probe tip that is commercially available ranges from 2mm, which can handle samples up to 5mL, to 25mm, which can handle samples up to 1litre. Pectin was extracted from pomegranate[18] and jackfruit peels,[19]and polysaccharide from rambutan peels[20] using a 20mm flat tip probe. Xu et al[21]. extracted pectin from grapefruit peel using 13mm and 25mm probes.

***Evaluation Parameter for Antioxidant property:***

For the study of the antioxidant effects in betel leaf extract, the inhibition of lipid peroxidation, superoxide generation and the activities of superoxide or hydroxyl radical scavenging are usually evaluated.[22]

**Table no.2 [22]**

| Sr. No. | Parameter | Observation |
|---|---|---|
| 1 | FeCl2- Ascorbic Acid stimulated Lipid Peroxidation in Rat Liver Homoganete | Significant increase in GMDA (Malandialdehyde) in test compared to normal control. |
| 2 | Inhibition of Xanthine Oxidase | The activity of xanthine oxidase was inhibited. |
| 3 | Free Radical Scavenger Activity | Antioxidant enyme such as SOD, P-ase, GSH, C-ase, phenolic,etc., lower the steady state concentration of ROS in cells & various tissue and there by prevent oxidative substance. |
| 4 | Enzyme assay from Herbal Extract by following method: | SOD activity is determined. |

| | | |
|---|---|---|
| 5 | (a) Assay for DPPH free radical scavenging activity | IC50 value denote the concentration of sample which is required to scavenge 50% DPPH free radicals. |
| 6 | Phospholipid Peroxidation | Inhibits peroxidation membrane lipid. |

***Free Radical Scavenger Activity:***

1. Samples are dissolved in 0.1m PBS to each concentration. Then 50ul of sample solution and 400ul working solution (xanthine/cytochrome c) are added to 530ul $H_2O$, mixed and then 20ul of 1units/ml of xanthine oxidase/ PBS are added, mixed vigorously and screened for 2 minutes at 550 nm.

2. Previous studies evaluated that the ESR technique is used to confirm the free radical scavenger activity.

3. Antioxidant enzymes which include super-oxide-dismutase (SOD), peroxidase (P-ase) and catalase (Case) enzyme,natural antioxidant include reduced glutathione (GSH), phenolics (tocopherols, flavonoids, phenolic acids, etc) contain activity of lowering the steady-state concentration of ROS in cells and many tissue and thus prevent oxidative substance.[23]

***Assay for DPPH free radical scavenging activity:***

1.DPPH (1,1-dipheny/-2 picryl- hydrazyl)is considered as a simple, rapid, stable, inexpensive and can be used widely, controlled method.[24]

2.DPPH can make stable free radicals in aqueous or ethanol solution.[23]Then, the free radical will undergo further reaction and thus create a stable product, while DPPH will accept an hydrogen or electron radical to become stable molecule.[25,26]

3. The antioxidant potency was previously evaluated through free radical scavenging by test samples, the change of optical density of DPPH radicals is monitored.[Lee et al,1998]

4. Reaction mixtures containing test samples (5ul of methanol: water 7:3 plant extract, dissolved in dimethyl sulphoxide (DMSO)] and 316umol/l DPPH ethanol solution (95ul, final DPPH concentration 300um0l/l) in 96- well microtitre plates are incubated at 37°C or 30min and absorbance was measured at 515nm. The % of inhibition by sample treatment is determined by comparison with a DMSO-treated control group.IC50 values denote the concentration of sample which is required to scavenge 50% DPPH free radicals.[23]

## Result & Discussion:

The data gathered shows that eugenol in betel leaf shows higher anti-oxidant activity by the mechanism of scevenging activity.From previous study, the result indicate that the presence of phenolic compound contributes to the antioxidant activity of the leaves and thus act as hydrogen donor, singlet oxygen quencher, heavy metal chelator, reducing agent.[27]The studies reported that increased production of free radicals in body escorts disturbance in antioxidand status, leading to cellular oxidative damage.[28] Eugenol acts as bioactive phytochemical against SOD (Superoxide dismutase) and MDA (Malondialdehyde) profiles in hypercholesterolemic rats.[29] The previous study reported that phenolic compound and flavonoids compound of betel leaf have both chemotherapeutic and chemo-presentive effects.[30,31,32] The study also result into showing anti-cancer activity of the P. betle extract due to high anti-oxidant activity.[33]

## Conclusion:

From the data gathered it was concluded that by Ultrasound Extraction method more eugenol extract is obtained. There are various methods for extraction but by ultrasound extraction the yield of eugenol is more. As study indicates that P. betle leaf extract has phenolic and flavonoid compound result into more anti-oxidant activity.Various formulation can also be prepared from betel leaf like cream , transdermal patches, suspension and many more.It was also seen that betel leaf can be used for ulcer treatment.

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