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Phytochemical screening and antioxidant activity of *Piper betle* leaf

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ABSTRACT

Piper betle, commonly known as betelvine, belongs to the family Piperaceae and is usually cultivated in Asian countries. Leaves of this plant have medicinal importance.The present study was conducted to determine the phytochemical composition and *in vitro* antioxidant activity of *Piper betle* leaf extracts. Aqueous and methanolic extracts were prepared from dried leaves. Preliminary phytochemical analysis confirmed the presence of various phytochemicals such as phenols, alkaloids, flavonoids, steroids etc. in these extracts. *In vitro* antioxidant activity of these extracts was investigated by DPPH-radical scavenging assay. Both extracts showed significant percentage radical scavenging activity.

Key words: *Piper betle, Phytochemicals, Antioxidant activity*

Introduction

The *Piper betle*, usually called as 'Paan', is an evergreen climbing plant with glossy, heart-shaped leaves. Betel leaves are extensively grown in South and Southeast Asia including India, Sri Lanka, Bangladesh, Malaysia, Thailand, and Taiwan. *Piper betle* is one of the invaluable medicinal plants where its leaves have been used for many medicinal purposes. It is a member of the Piperaceae family. The betel leaves are aromatic with varied taste, i.e. from sweet to pungent, due to the presence of its essential oils. The betel leaves are mainly used as a mouth freshener and is also well known for curing many communicable and non-communicable diseases like cold, cough, bronchial asthma, rheumatism, stomachalgia and used to treat other diseases like bad breath, boils and abscesses, conjunctivitis, constipation, swelling of gums, cuts and injuries (Gundala *et al.,* 2014).

There have been a number of studies on the analysis of phytochemical components of the *Piper betle* as well as the extraction and isolation of interesting compounds from betel plants (Hussain *et.al.,* 2014; Nguyen *et al*., 2020). However, the composition of the plant extracts varied strongly depending on geographical origin as well as extraction techniques (Rintu *et al*., 2015). *Piper betle* leaves contain various biologically active compounds which are responsible for antioxidant activity (Sripradha, 2014). Production of free radicals and other reactive species in cells and body tissues has been linked to ageing and several diseases in human being. Antioxidants play an important role in scavenging free radicals and/or chain breaking of the oxidation reaction chemically. (Jayalakshmi *et al*., 2015).

Currently, there is growing interest, both in the industry and in the scientific research, for medicinal plants because of their antioxidant properties. These properties are due to many active phytochemicals

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including phenols, terpenoids, tannins, saponins, alkaloids, flavonoids, steroids, etc. Scientific research on the betel leaves reveals that it possesses many beneficial bioactivities and its extract has a great potential in developing commercial products.

Materials and Methods

Collection of sample

Fresh and healthy leaves of *Piper betle* were collected from the betelvine farm in Kelwa village of Palghar district, Maharashtra.

Preparation of extracts

Betel leaves were washed under distilled water, shade dried and ground to get fine dry powder. Aqueous and methanolic extracts were prepared from 15g powder of dried betel leaves and 300ml solvents, through Soxhlet extraction method. Then collected extracts were evaporated to dryness and stored in the refrigerator at 4 ºC for further analysis. (Banik *et al*., 2020)

Preliminary Phytochemical Analysis

Preliminary phytochemical analysis of both extracts was carried out by standard methods (Shaikh and Patil, 2020). All the tests were performed in triplicates.

Test for phenols: 2ml extract was mixed with 2 ml of 2% $FeCl_3$. A blue-green or black coloration indicates the presence of phenols.

Test for Alkaloids: 2 ml extract was mixed with 2ml of 1% HCl and heated gently. Few drops of Wagner's reagent were added to the solution. A reddish brown precipitate indicates the presence of alkaloids.

Test for flavonoids: 2ml extract was mixed with 2ml of 2% NaOH. An intense yellow colour indicates presence of flavonoids.

Test for saponins: 1ml extract was mixed with 2 ml distilled water and shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Test for Glycosides: 2 ml Extract was mixed with 2 ml glacial acetic acid and one drop of 5% $FeCl_3$ was added to the solution followed by few drops of concentrated H_2SO_4. Reddish brown colour at junction of the two liquid layers indicates the presence of glycosides.

Test for Steroids: 2 ml extract was mixed with 2ml chloroform and 2 ml concentrated H_2SO_4 was added to the solution. Appearance of reddish colour in chloroform layer indicates presence of steroids.

Test for Terpenoids: 2 ml extract was mixed with 2 ml of chloroform and evaporated to dryness. 2 ml of concentrated H_2SO_4 was added to the solution and heated for about 2 minutes. Development of grayish color indicates the presence of terpenoids.

Determination of *In vitro* Antioxidant activity

The free radical scavenging activity of betel leaf extracts was measured *in vitro* by DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) -radical scavenging assay. (Karak *et al*., 2019).

1mg/ ml sample was used for the analysis. 1ml sample extract was added to 3ml of 0.1mM solution of DPPH. Reference containing 3ml DPPH and 1ml methanol was run simultaneously. Following incubation in dark at room temperature for 30 minutes, absorbance of the reference and sample were determined colourimetrically at 520nm using methanol as a blank. 1mg/ml ascorbic acid served as a positive control. Each sample was tested in triplicate.

The percentage free radical scavenging activity was calculated from obtained absorbance by the equation:

% free radical scavenging activity = [(O.D of Reference - O.D of test) / O.D of Reference] x 100

Results and Discussion

The phytochemical tests and in vitro antioxidant activity of aqueous and methanol extracts from *Piper betle* leaves were performed and the results are presented in Table 1 and 2 respectively.

In the current investigation both aqueous and methanolic extracts showed the presence of phenols,

Table 1. Phytochemical analysis of betel leaf extracts

Sr. No.	Phytochemical	*Piper betle* Aqueous extract	*Piper betle* Methanolic extract
1	Phenols	+	+
2	Flavonoids	+	+
3	Saponins	+	-
4	Glycosides	+	+
5	Steroid	+	+
6	Terpenoids	+	+
7	Alkaloids	+	+

Note: '-' indicated absent, '+' indicated present

Table 2. *In vitro* antioxidant activity of of betel leaf extracts

Sr. No.	Type of extract	Percentage free radical scavenging activity
1	*Piper betle* Aqueous extract	63.51%
2	*Piper betle* Methanolic extract	71.34%
3	Ascorbic acid standard	68.32%

flavonoids, glycosides, steroids, terpenoids and alkaloids. However saponins were absent in methanolic extract but present in aqueous extract. Raghavan *et al*. (2018) have also reported the presence of alkaloids, phenols, flavonoids and terpenoids in methanol extract of "Thulasivettila" cultivar of *Piper betle*. from Thiruvananthapuram district, Kerala. Johri *et al*. (2017) have identified alkaloids, steroids, saponins, phenolics and flavonoids in aqueous and methanol extracts of *Piper betle* leaves collected from Gwalior, M.P.

Current study showed that methanolic extract has higher radical scavenging activity compared to aqueous extract as well as standard ascorbic acid. Sarma *et al.* (2018) have reported higher antioxidant activity in alcoholic extract than aqueous extract of Meetha paan and Banarasi paan. Jayalakshmi *et. al*. (2015) have also reported higher antioxidant activity in methanolic extract of ambadi variety of *Piper betle* from Mysore, Karnataka.

The findings of present study showed that both aqueous and methanolic extracts of *Piper betle* leaves are rich in phytochemicals and also exhibit free radical scavenging activity against DPPH.

Conclusion

The present study demonstrated that methanolic extract of *Piper betle* cultivar from Kelwa village has significant free radical scavenging activity against DPPH, which revealed that the methanol is more suitable in extracting phyto chemicals of betel leaves than water. Presence of phyto chemicals in these extracts may contribute to the antioxidant activity. Hence, these betel leaves can be further exploited for their antioxidant potential.

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