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Phytochemical Analysis and Antioxidant Property of *Premna latifolia*

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Abstract

Plants have been used for centuries as a remedy for human diseases because they contain phytochemical components of therapeutic values. Hexane, Ethyl acetate and Ethanolic extract of *Premna latifolia* were investigated for Phytochemical constituent and Antioxidant activity. The phytochemicals were investigated using the standard chemical methods. Further the study was extended by analyzing the antioxidant potential using DPPH method. The results showed that ethanolic extract showed significant number of phytoconstituents and better radical scavenging activity at the concentration of 75µg/ml.

Keywords

Premna latifolia, DPPH, Antioxidant, Phytochemicals

1. Introduction

Premna latifolia belongs to the family verbenaceae, widely distributed in tropical and subtropical and coastal areas. The leaves are diuretic in nature and is used as a folk medicine for treating dropsy[1]. *Premna latifolia* possesses anti-inflammatory activity in the animal models[2]. *Premna latifolia* bark is applied to cure boils. The leaves are diuretic in nature and is used as a folk medicine for treating dropsy[3]. *Premna latifolia* possesses anti-inflammatory activity in the animal models. *Premna latifolia* bark is applied to cure boils. Traditionally it has been used in the treatment of hepatic disorders[4], antioxidant[5] and anticancer activity[6].

Herbal medicines from the plants act as a primary sources for the discovery of new drugs. Phytochemicals are defined as bioactive nonnutrient plant compounds reducing the risk of major chronic diseases[7]. The significant phytochemicals such as alkaloids, tannins, glycosides, flavonoids, steroids, tannins etc, constitute of chemical agents for the approach of new anti-infective agents. Antioxidants are the compounds which help to delay or inhibit the oxidation of lipids and other molecules through the inhibition of either initiation or propagation of oxidative chain reactions[8]. Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia, reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS [9] , [10].

2. Materials and Methods

2.1. Collection of Plants

Plants for this study were collected from Chinnapaliyampattu villege, Tiruvannamalai district, Tamilnadu and were authentified by Dr. Rathna kumar, Department of plant biotechnology, Presidency college, Chennai-05.

2.2. Preparation of Extracts

Collected plants were dried at room temperature and ground to make fine powder. 20gm of plant powder was well dissolved in 100ml of solvents (Hexane, Ethyl acetate and Ethanol) (ratio 1:5). The suspension was filtered by using Filter paper of pore size 0.2µm. The filtrate was then air dried and extracts were collected in sterile vials for further use.

2.3. Phytochemical Tests

The phytochemical test of these extracts was performed using the method adopted by Harborne[11] and Sofowora[12].

2.3.1. Test for Carbohydrates (Molisch's Test)

To 2ml of plant extract, 1ml of Molisch's reagent and a

few drops of concentrated sulfuric acid were added. Presence of purple or reddish color indicates the presence of carbohydrates.

2.3.2. Test for Tannins (Ferric Chloride Test)

To 1ml of plant extract, 2ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

2.3.3. Test for Saponins (Frothe's Test)

To 2ml of plant extract, 2ml of distilled water was added and shaken in a graduated cylinder for 15minutes lengthwise. Formation of a 1cm layer of foam indicates the presence of saponins.

2.3.4. Test for Flavonoids (Shinoda Test)

To 2ml of plant extract, 1ml of 2N sodium hydroxide was added. Presence of yellow color indicates the presence of flavonoids.

2.3.5. Test for Alkaloids (Mayer's Test)

To 2ml of plant extract, 2ml of concentrated hydrochloric acid was added. Then a few drops of Mayer's reagent were added. The presence of green color or white precipitate indicates the presence of alkaloids.

2.3.6. Test for Quinones

To 1ml of extract, 1ml of concentrated sulfuric acid was added. Formation of red color indicates presence of Quinones.

2.3.7. Test for Glycosides (Molisch's Test)

To 2ml of plant extract, 3ml of chloroforms and 10% ammonia solution was added. Formation of pink color indicates presence of glycosides.

2.3.8. Test for Cardiac Glycosides (Keller–Kiliani Test)

To 0.5ml of extract, 2ml of glacial acetic acid and a few drops of 5% ferric chloride were added. This was under layered with 1 ml of concentrated sulfuric acid. The formation of brown ring at the interface indicates presence of cardiac glycosides.

2.3.9. Test for Terpenoids (Salkowski Test)

To 0.5ml of extract, 2ml of chloroform was added and concentrated sulfuric acid is added carefully. Formation of red brown color at the interface indicates presence of terpenoids.

2.3.10. Test for Triterpenoids

To 1.5ml of extract, 1ml of Libemann –Buchard Reagent (aecticanhydride+ concentrated sulfuric acid) was added. Formation of blue green color indicates presence of triterpenoids.

2.3.11. Test for Phenols (Ferric Chloride Test)

To 1ml of the extract, 2ml of distilled water followed by a few drops of 10% ferric chloride was added. Formation of blue or green color indicates presence of phenols.

2.3.12. Test for Coumarins

To 1 ml of extract, 1ml of 10% NaOH was added. Formation of yellow color indicates presence of coumarins.

2.3.13. Steroids and Phytosteroids (Libermann-Buchard Test)

To 1ml of plant extract equal volume of chloroform is added and subjected with a few drops of concentrated sulfuric acid appearance of brown ring indicates the presence of steroids and appearance of the bluish brown ring indicates the presence of phytosteroids.

2.3.14. Phlobatannins

To 1ml of plant extract few drops of 2% HCL was added appearance of red color precipitate indicates the presence of phlobatannins.

2.3.15. Anthraquinones (Borntrager's Test)

To 1ml of plant extract few drops of 10% ammonia solution were added, appearance pink color precipitate indicates the presence of anthraquinones.

2.3.16. Antioxidant Activity

The antioxidant activity of Plant extracts was determined by, the DPPH(1, 1-diphenyl-2-picryl-hydrozyl) in vitro method.

2.3.17. DPPH Free Radical Scavenging Activity

The antioxidant activity of Hexane, Ethyl acetate and Ethanolic extracts of *Premna latifolia* and the standard compound BHT was measured in terms of hydrogen donating radical scavenging ability using the stable DPPH method[13]. 1ml of extract was added to 3.7mL of methanol solution. After centrifugation, the supernatant is collected 200µml of DPPH solution is added. Kept in the dark for 45 min and the resulting decrease in absorbance at 517 nm were recorded against blank using a UV-Vis Spectrophotometer. The radical scavenging activity on DPPH was expressed as, % DPPH radical-scavenging = [(Absorbance of control - Absorbance of test Sample) / (Absorbance Of control)] x 100.

3. Results

The preliminary phytochemical screening of *Premna latifolia* showed the presence of plant components such as carbohydrates, flavonoids, quinones and coumarins in hexane extract, carbohydrates, tannins, flavonoids, cardiac glycosides, phenols and coumarins in ethyl acetate extract and carbohydrates, tannins, flavonoids, quinones, cardiac glycosides phenols and coumarins in ethanol extract.(Table 1).

Free radical scavenging activity(DPPH)

The stable free radical scavenging activity by the DPPH method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific plant extracts. Figure 2 indicates the percentage of free radicals scavenging activity in various extractions with different concentrations 25µg, 50µg and 75µg of *Premna latifolia*. In this study percentage

inhibition of free radicals was carried out with different extractions of selected plants. Ethanol extract with 75µg concentration gives higher percentage (57.03%), Hexane extract showed moderate activity of 47.31% and Ethyl acetate extract showed least activity of 46.36% of free radical scavenging activity. The free radical scavenging activity increases with increase in concentration(Table 2, Fig.1). The percentage inhibition of control was found to be 81.68% which showed higher activity than the extract.

Table 1. *Phytochemical analysis of Premna latifolia.*

S.No	Phytochemical Tests	Test performed	Hexane Extract	Ethyl Acetate Extract	Ethanol Extract
1.	Carbohydrates	Molisch'stest	+	+	+
2.	Tannin	Ferric chloride test	-	+	+
3.	Saponin	Frothe's test	-	-	-
4.	Flavonoids	Shinoda test	+	+	+
5.	Alkaloids	Mayer's test	-	-	-
6.	Quinones	-	-	-	+
7.	Glycosides	Molisch's test	-	-	-
8.	Cardiac glycosides	Keller – Kiliani test	+	+	+
9.	Terpenoids	Salkowski test	+	-	-
10.	Phenols	Ferric chloride test	+	+	+
11.	Coumarins	-	+	-	-
12.	Steroids	Libermann – Buchard test	-	+	-
13.	Phlobotanins	-	-	-	-
14.	Anthraquinones	Borntrager's test	-	-	-

Table 2. *DPPH assay of Premna latifolia against different extracts.*

Concentrations (µg)	Control	% of Inhibition			
		Hexane	Ethyl acetate	Ethanol	BHT
25	0.9593	36.07± 0.97	36.95± 0.96	46.55± 0.98	72.36 ± 1.64
50	0.9593	41.15 ± 0.62	41.97 ± 0.53	51.72 ± 0.76	76.23 ± 0.85
75	0.9593	47.31± 0.43	46.36± 0.35	57.03± 0.54	81.68 ± 0.61

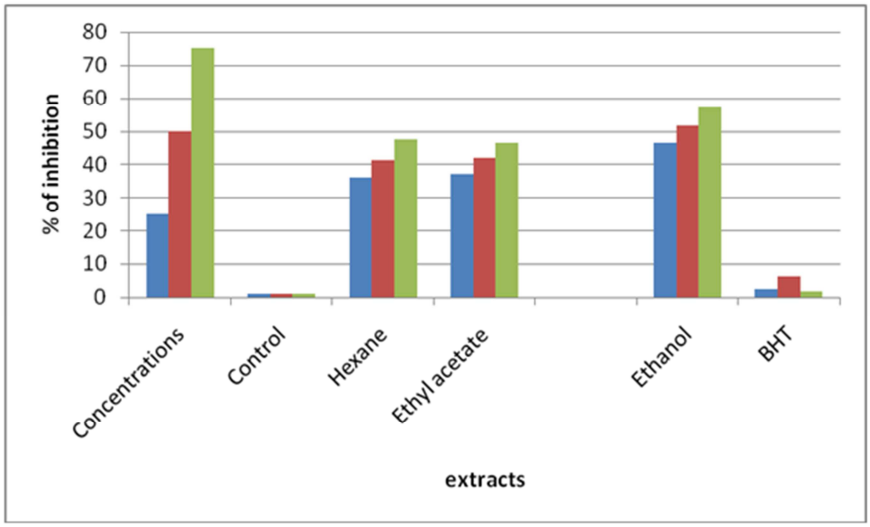

Figure 1. *Antioxidant activity of Premna latifolia by DPPH assay.*

4. Discussions

The results of the present study reveals that the ethanolic extract of *Premna latifolia* showed maximum number of components such as Carbohydrate, tannin is used for the treatment of skin eruption, antimicrobial activity and bowel condition, flavonoides possess wound healing activity due to the astringent, antioxidant and antimicrobial properties which appear to be responsible for wound contraction and elevated rate of epithelisation[14], Quinones, Cardiac glycosides, phenols was found to be toxic to the growth and development of pathogens and Coumarins in the ethanolic extract when compared to other solvents. Previous study reported that the stem bark shows the presence of iridoid glucosides and geniposidic acid[15], [16]. Yet another report says that stem powder of *Premna latifolia* shows the presence of carbohydrates, proteins, phenols, oils, fats, terpenoids, steroids, saponins, flavonoids, alkaloids and tannins[17], [18]. The DPPH scavenging of the stem extract of *Premna latifolia* was studied as described by Singh[19], [20]. Thus the present work also correlates with the aforesaid studies. The significant concentration of antioxidant in the ethanolic

extract is due to phytoconstituents. However the extract was not effective as the standard antioxidant BHT. Thus the ethanolic extract showed maximum antioxidant activity when compared to other solvents. Hence the ethanolic extract can act as a antioxidant activity and can protect the cells from the free radicals.

5. Conclusion

The present study suggests that the *Premna latifolia* have maximum number of bioactive components and higher amount of antioxidant potential in the ethanolic extract, therefore the ethanolic extract may act as a significant activity and can be further analysed for many pathogenic disorders as well as may be helpful in future for preventing or slowing the progress of diseases involved. However it is obvious that less information was available further in order to explore this plant more researchers should be carriedout.

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