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Full Length Original Research Paper

Antioxidant activity and Phytochemical Analysis of D*atura metel*

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Abstract:
The objective of this study is to determine antioxidant activity and phytochemical analysis of Datura metel by using four different types of solvents **n- hexane, ethyl acetate, chloroform,** and methanol. Sequencing extract was prepared. The antioxidant activity of different solvent extracts from the leaves of *Datura metel* was tested by Total phenolic content, DPPH scavenging activity, Hydroxyl radical scavenging activity, Reducing power assay, and β –carotene bleaching activity. The antioxidant activity was performed at four different concentrations ranging from 25-100mg/ml.The phytochemical analysis of all crude extracts showed a positive result for alkaloids, saponins, flavonoids, tannins, glycosides, aminoacids and phlobatannins, whereas steroids and terpenoids are absent. Chloroform extract exhibited highest antioxidant activity with increase in concentration.

Keywords: Datura metel, antioxidant activity, DPPH, β –carotene bleaching activity.

Datura metel is a medicinal herb. The name of the Datura comes from Sanskrit Dustura[1]or Dahatura. *Daturametel* is a flowering plant and grows upto 3 feet high. The leaves of *Datura metel* are about 10-20 cm long and 5-18 cm broad. Its leaves are covered with short and soft grayish hairs. It is commonly found in Asia, England, Africa, India and other tropical & subtropical regions.[2]

Primarily this plant is used as an intoxicant and hallucinogen[3][4]. The leaves and seeds of Datura species were rich in alkaloids, including atropine, scopolamine and hyoscyamine[5]. The phytoconstituents such as flavonoids, phenols, tannins, saponins, aminoacids and sterols are found in *Datura metel*[4].

Especially in India, it is used for the treatment of epilepsy, hysteria, heart diseases, cough, convulsions, diarrhea, skin diseases, etc[6][7]. *Datura metel* was also been used for its anesthetic or pain- killing properties. Several scientific studies have been reported on antioxidant and phytochemical screening of ethanol and hydro alcoholic crude extract[8] [9]*; however,* this study investigate the phytochemical composites and antioxidant activity of different types of solvent extracts (non polar solvent: n-Hexane & polar solvent: Ethyl acetate, Chloroform and Methanol).

Materials and Methods:

Collection of leaves:

The leaves were collected from the field of Vellore. The collected leaves were washed with water to remove dust and then washed leaves were shade dried for 40 days. After drying the leaves were ground into powder form using a mixer.

Extract preparation:

Extraction procedure was done according to the method of Vikrant Arya *et al.*[10].Sequencing extracts were prepared by using four solvents n-Hexane, Ethyl acetate, Chloroform and Methanol.

Preliminary phytochemical screening:

The phytochemical tests were done for analyzing different chemical groups present in the extracts. This screening was done according to the method of Mehta Kavit *et al.*[11]

Test for alkaloids:

Three ml of extract was added to 1% HCl and then allowed to steam bath. Few drops of Mayer & Wagner's reagent was added to the mixture. Turbidity indicates the presence of alkaloids.

Test for flavonoids:

To 1 ml of extract, 1 ml of 10% lead acetate was added. Formation of yellow precipitate showed the presence of flavonoids.

Test for saponins:

Two ml of extract was shaken vigorously with 5 ml of distilled water and warmed. The formation of stable foam indicates the presence of saponins.

Test for tannins:

To 2 ml of extract, 2 ml of distilled water was taken and stirred. Few drops of ferric chloride solution were added. The formation of green precipitate showed the presence of tannins.

Test for steroids:

Two ml of extract was dissolved in chloroform; 2 ml of concentrated sulphuric acid was added to the mixture. Red color formation indicates the presence of steroids.

Test for terpenoids:

To 1 ml of extract, 0.5 ml of acetic anhydride was added. And then few drops of concentrated sulphuric acid were added to the mixture. Bluish green precipitate showed the presence of terpenoids.

Test for phlobatannins:

Two ml of extract was hydrolysed with 1% HCl and mixture was boiled for few minutes. Deposition of red precipitate indicates the presence of phlobatannins.

Test for glycosides:

Two ml of extract was dissolved in chloroform and 2 ml of acetic acid was added to the mixture. The solutions were cooled and then add few drops of sulphuric acid. A color change from blue to green indicates the presence of glycosides.

Test for amino acids:

One ml of extract was treated with few drops of Ninhydrin reagent. A purple color indicates the presence of amino acids.

Antioxidant activity:

Total phenolic content:

Total phenolic content of *Datura metel* solvent extracts was determined (*Meda et al*)[12].Gallic acid was used as standard. Four concentrations 25, 50, 75,100 mg/ml of solvent extracts were taken in 4 test tubes. 2.5 ml of 10 – fold diluted Folin- Ciocalteu reagent was added and 2.0 ml of 7.5% sodium carbonate was added. The reaction mixture was incubated at 40^0 C for 30 minutes. Finally, absorbance was read at 760 nm. This was done in triplicate.

DPPH radical scavenging:

The DPPH assay was done according to the method of Rivero-perez *et al*[13] .The DPPH scavenging effects of the different extracts n-Hexane, Ethyl acetate, Chloroform& Methanol were determined. Ascorbic acid was used as a standard. The blank contained 1ml of distilled water. 1 ml of extract was taken at four concentrations of 25, 50, 75, 100 mg / ml in 4 test tubes. 1 ml of 0.2mM DPPH ethanol solution was added. The mixture was vortexed vigorously for 1 minute and kept in an dark for 60 minutes. The

absorbance of all samples was measured at 517 nm. This was done in triplicate. The percentage inhibition was calculated using the formula,

% Inhibition =$A_{control} - A_{extract} / A_{control} \times 100$.

Hydrogen peroxide radical scavenging activity:

The hydrogen peroxide radical scavenging activity was determined by the Ruch *et al*[15] method. 1 ml of extract at four concentrations 25, 50, 75,100 mg/ml was taken in 4 test tubes. 0.6 ml of hydrogen peroxide (40mM) was added with phosphate buffer. The samples were incubated at 30^{0}C for 10 minutes. After incubation, absorbance was determined at 230 nm against phosphate buffer as a blank. L- Ascorbic was used as standard. This was done in replicate. The percentage inhibition was calculated using the formula:

% of inhibition = $(A_1-A_2)/A_1 \times 100$.

Whereas, A_1 – absorbance of the H_2O_2 without extract.

A_2 – absorbance of the sample with extract.

β-carotene bleaching activity:

The β carotene activity was done according to the method of Wettasinghe *et al* [16]. 3 ml of β-carotene solution (5 mg of β carotene/50 ml of chloroform) was added to 40 mg of linoleic acid and 400 mg of Tween 20. Then, the mixture was mixed well and dried. To the dried mixture, 100 ml of distilled water was added in order to form β-carotene linoleic acid emulsion. 1 ml of solvent extract was taken in 4 test tubes and 1.5 ml of emulsion was added. Then, the mixture was incubated in water bath at 50^{0}C for 60 minutes. Alpha- tocopherol was used as standard. This was done in replicate. Finally, the absorbance was read at 470 nm. Antioxidant activity (AA %) of *D. metel* extract was calculated by using the formula:

AA (%) = 100 ($DR_C - DR_S$) / DR_C.

Whereas, AA= antioxidant activity.

DR_C= degradation rate of the control.

DR_S= degradation rate of the sample ((a/b)/60) a=initial absorbance of the sample. b=absorbance after 60 min of incubation.

Reducing power:

The ability of extracts to reduce iron (III) was determined by the method of Yildirim *et al.,* [14]. Four concentrations 25, 50, 75,100 mg/ml of *Datura metel* solvent extracts were mixed with 2.5 ml of phosphate buffer and 2.5 ml of potassium ferricyanide. Then, the mixture was incubated at 50^{0}C for 30 minutes. 2.5 ml of Trichloroacetic acid was added and the mixture was centrifuged at 3000 rpm for 10 minutes. 2.5 ml of supernatant solution was taken and mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride. L-ascorbic acid was used as standard. Finally, the absorbance of all samples was measured at 700 nm. This was done in replicate

RESULT AND DISCUSSION:

Phytochemical screening:

Table 1: Phytochemical analysis of hexane, ethyl acetate, chloroform and methanol crude extract from leaf of *Datura metel.*

	Hexane	Chloroform	Ethyl acetate	Methanol
Alkaloid	+	+	+	+
Tannins	+	+	-	+
Saponins	+	+	+	+
Flavonoids	-	+	+	+
Steroids	-	-	-	-
Terpenoids	-	-	-	-
Phlobatannins	-	+	-	+
Glycosides	-	+	+	-
Amino acid	+	+	+	+

+ = Presence; - = Absence.

Phytochemical screening result shows the flavonoids, tannins, alkaloids, glycosides, aminoacids, saponins, and phlobatannins were present in leaf crude extracts of *Datura metel,* whereas steroids and terpenoids were absent in all the extracts (Table1).However, chloroform crude extracts showed positive test for alkaloids, flavonoids, tannis, glycosides, aminoacids, saponins, phlobatannins but steroids and terpenoids are absent. Methanol crude extracts shows the presence of alkaloids, tannins, saponins, flavonoids, phlobatannins, and aminoacids but glycosides, steroids and terpenoids were absent. In ethyl acetate crude extracts the chemical compounds such as flavonoids, saponins, alkaloids, glycosides, aminoacids were present but tannis, phlobatannins, steroids and terpenoids were absent. Hexane crude extracts shows the presence for alkaloids, tannins, aminoacids, and saponins whereas absent in all other screening test. Phytochemical constituents which are present in plant samples are known to be biologically active compounds and they are responsible for different activities such as antimicrobial, antioxidant, antifungal, anticancer and antidiabetic[17] .Different phytochemicals have been found to possess a wide variety of pharmacological activities, which may help in protection against chronic diseases. Tannins, glycosides, saponins, flavonoids, and aminoacids have hypoglycemic and anti-inflammatory activities. Terpenoids, and steroids shows analgestic properties and central nervous system (CNS) activities. Saponins are involved in plant defense system because of their antimicrobial activity[18] and also possess hypocholesterolemic and antidiabetic properties. The most effective bio active compounds are alkaloids, aminoacids and saponins these were found in all four types of crude extracts. Flavonoids were found in methanol, chloroform and ethylacetate except hexane extracts. Chloroform and methanol extracts shows the presence of majority phytoconstituents. Many reports are available on flavanoid groups which exhibiting high potential biological activities such as antioxidant, anti-inflammatory, antiallergic reactions[19-22] .

Antioxidant activity:

Total phenolic content:

The amount of total phenolic compounds of crude extract was determined by using linear gallic acid($y=8.7231X+0.087$;$r^2=0.9971$).The total phenolic content ranges from 0.53-3.99mg/ml. Among different types of extracts chloroform shows highest antioxidant activity whereas n-hexane shows lowest antioxidant activity. In many plants, phenolic compounds shows secondary metabolites with antioxidant and antibacterial activites. In many countries, 80% of people make use of medicinal plants for maintaining good health because of antioxidant property. Most medicinal plants contain higher phenolic compounds such as monophenols and polyphenols. Usually in many plants leaf shows the higher phenolic content [23] [24].Many studies have proved that the phenolic content in the plants are associated with their antioxidant activities, probably due to their redox properties, which allow than to act as reducing agents and hydrogen donors[25].

DPPH radical scavenging:

The photometric evaluation of the antioxidant activity of chloroform extract of *Datura metel* leaves shows good antioxidant capacity.The inhibition percentage of different extracts ranges from 5.97 to 53.2mg/ml. In all extracts , Chloroform showed maximum inhibition whereas n-hexane

showed minimum inhibition. Chloroform extract contain highest amount of total phenolics, was found to be most effective radical scavenger followed by ethylacetate, methanol & n-hexane extract. DPPH is used to evaluate the free radicals[26].Free radicals are involved in the process of lipid per oxidation which is considered as a major role in chronic diseases[27].All extracts from *Datura metel* leaves exhibited a significantly greater hydroxyl radical scavenging activity than the ascorbic acid.

Hydrogen peroxide radical scavenging activity:

Oxidative DNA damage is occur is due to presence of H_2O_2 in the cell, hence removing hydrogen peroxide is very essential for antioxidant defense in cells. Reactive agent of hydrogen peroxide sometimes causes cell death due to the production of hydroxyl radical within the cell. The overall inhibitory activity of solvent extracts against hydrogen peroxide can be presented in the following order: L-Ascorbic acid >Chloroform>Methanol>Hexane>Ethylacetate. Chloroform exhibit highest inhibition(79%) and Ethyl acetate exhibit lowest inhibition(42%).The leaf extract might contain primary antioxidant compounds, which are able to react with free radicals, especially hydroxyl radicals thereby ending the radical-chained reaction& stop the formation of hydroperoxides[28] [29].

β-carotene bleaching activity:

β carotene bleaching activity was determined through the linear a-tocopherol standard curve($y=16.988X+3.342$; $r^2=0.9786$). During incubation linoleic acid produces hydroperoxides. Due to the presence of hydroperoxide, rapid discoloration of β carotene occur. However, hydroperoxides formed in this system can be neutralized by the antioxidants from the extracts. In all extracts, chloroform shows highest antioxidant activity(4.58%mg/ml) whereas hexane shows lowest antioxidant activity(0.56%mg/ml).The yield of all crude extract from *D. metel* arranged in the following order: a- tocopherol > Chloroform > Methanol > Ethylacetate > Hexane.

Reducing power:

Many studies have indicated that the electron donation capacity of compounds is related with antioxidant activity. The reduction ability was estimated through Fe^{3+} - Fe^{2+}. Chloroform exhibits maximum reducing activity and hexane exhibits minimum activity. All extracts showed electron donation capacity. The higher absorbance value indicated that higher antioxidant activity. Chloroform extract contain highest amount of total phenolics and it is the most potent reducing agent. Relation between iron (III) reducing activity and total phenol content have been reported in the literature [30]; however the correlation may not be always linear[31].

Figure 1: Total phenolic concentration of hexane, ethyl acetate, chloroform,methanol extracts of *Datura metel* leaves.

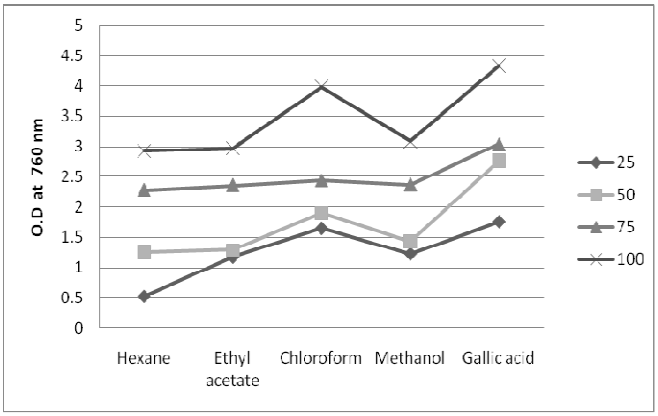


Figure 2: DPPH radical scavenging activities of hexane, ethyl acetate, chloroform, methanol extracts of *Datura metel* leaves.

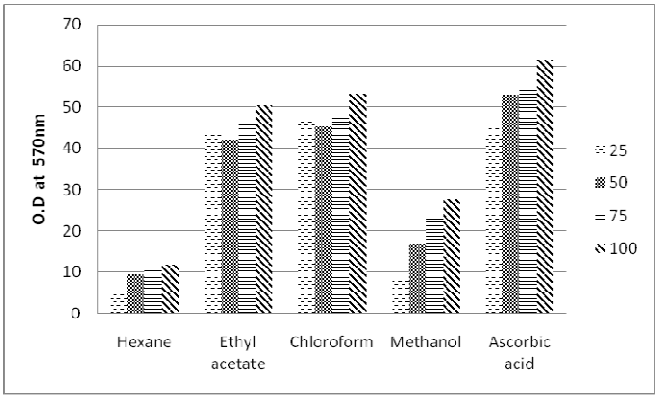


Figure 3: Hydrogen peroxide radical scavenging activity of hexane, ethyl acetate, chloroform, methanol extracts of *Datura metel* leaves.

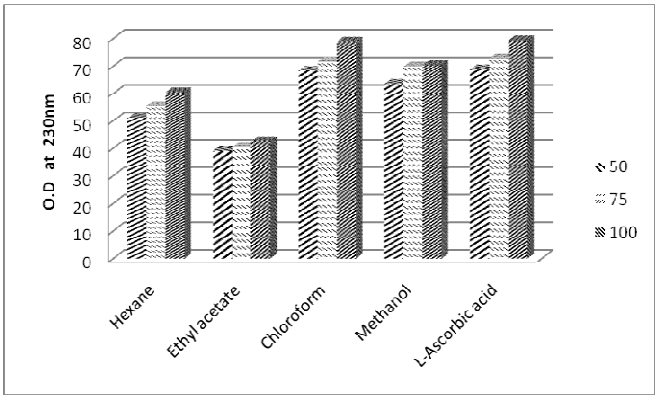


Figure 4: Antioxidant activity of *Datura metel* extracts through β carotene bleaching assay.

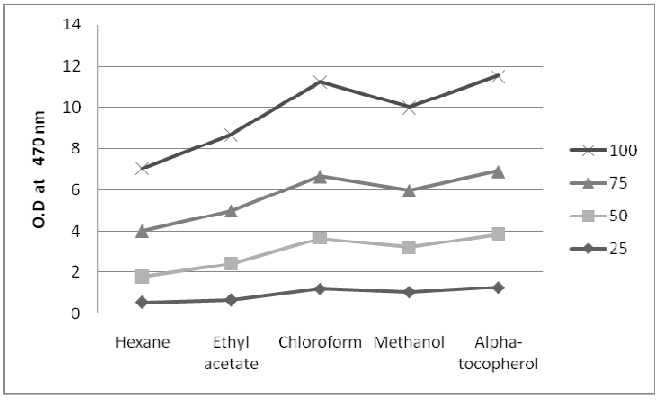
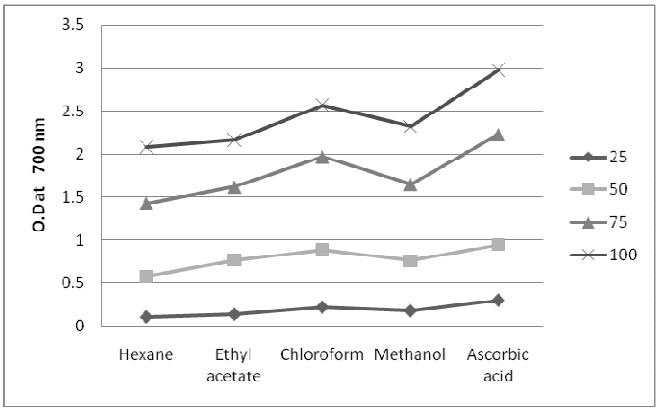


Figure 5: Reducing powers of hexane,ethylacetate,chloroform,methanol extracts of *Datura metel* leaves



Conclusion:

High antioxidant activity was observed in chloroform extracts of *Datura metel* when compared to other extracts. The extracts of *D. metel* show the presence of secondary metabolites such as alkaloids, saponins, glycosides, phlobatannins, tannis, aminoacids and flavonoids. Chloroform contains highest amount of phenolic content and also exhibit strongest antioxidant capacity in the entire assay used. According to the phenolics result chloroform crude extract could be used as natural antibiotics for different diseases. The bioactive compounds from *D. metel* serve as good phytotherapeutic agent.

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