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# PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF VARIOUS EXTRACTS OF *CLERODENDRU M PANICULATUM LINN.*

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## ABSTRACT

*Clerodendrum paniculatum* Linn. of Verbenaceae family is used for the treatment of various diseases like asthma, typhoid, cancer and jaundice. Phytochemical screening revealed the presence of alkaloids, terpenoids, sterols, flavanoids, phenolics, carbohydrates and glycosides. The investigation was undertaken to appraise the antioxidant protection of different extracts of *Clerodendrum paniculatum* Linn. using beta carotene bleaching assay and nitric oxide radical scavenging assay. It was found that ethyl acetate extract has good free radical scavenging activity than other extracts by both methods. In these testing a significant correlation existed between concentration of the extracts and percentage inhibition of free radicals. The results clearly indicate that *Clerodendrum paniculatum* Linn. Is effective against free radical mediated diseases.

**KEYWORDS:** Verbenaceae, *Clerodendrum paniculatum* Linn., beta carotene bleaching assay, nitric oxide radical scavenging assay.

## INTRODUCTION

Polyphenolic compounds belong to a large heterogenous group of secondary plant metabolites which are usually widespread in the plant kingdom and have various application

in food, cosmetic and pharmaceutical industries.[1] Free radical are produced in the oxidation reactions which can start chain reactions and that damage cells. Antioxidants terminate these chain reactions by being oxidized themselves. The phenolic comounds has antioxidant capacity due to the redox properties which act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators.[2] Antioxidant based drug or formulation is widely used for the prevention and treatment of complex diseases like atherosclerosis, neurodegenerative diseases, stroke, diabete, Alzhemer's disease and cancer during the last three decades.[3]

This has attracted a great deal of research in natural antioxidants, The majority of the active antioxidant compounds are flavones, flavanoids, isoflavones, anthocyanins, coumarins, lignans, catechins and isocatechin.[4]

The genus *Clerodendrum* L. is widely distributed in tropical and subtropical regions of the world. More than 500 species of the genus are identified. Ethno medical importance of various species of *Clerodendrum* species have been reported in various indigenous systems of medicines and as folk medicines. The genus is being used as medicines especially in Indian, Chinese, Thai, Korean, Japanese systems of medicines for the treatment of various life threatening diseases such as typhoid, syphilis, cancer, jaundice and hypertension. The powder /paste form and the various extracts of roots, stems and leaves are reported to be used as medicines for the treatment of asthma, cataract, malaria, diseases of blood, skin and lung. The major chemical components reported from the genus are phenolics, steroids, di- and tri terpens, flavonoids, volatile oils etc.[5,8]

There is a growing interest all over the world for discovering the untapped reservoir of medicinal plants. Hence the present study was aimed at measuring the relative content of phenolics and antioxidant capacities of *Clerodendrum paniculatum* Linn. leaves.

## MATERIAL AND METHODS

### Collection of plant materials

The fresh leaves of *Clerodendrum paniculatum* Linn. were collected from Ettumanoor, Kottayam (dist), Kerala and authentified by the botanist Mr. Joby Paul, School of Environmental Science, M. G University, Kottayam. voucher specimen (No: CPS 12/09) was deposited in the Herbarium of same department.

**Extraction**

Extraction of *Clerodendrum paniculatum* Linn. leaves were carried out by solvents of increasing polarity such as petroleum ether, chloroform, ethyl acetate, ethanol and water.

**Extraction with petroleum ether**

Extraction of *Clerodendrum paniculatum* Linn. leaves with; petroleum ether was carried out by simple maceration technique. 700g of *Clerodendrum paniculatum* Linn. leaves was taken, size reduced and extracted with 2L of petroleum ether in amber coloured bottle with intermittent shaking for 5 days. After 5 days, extract was decanted off and allowed to evaporate at room temperature in an evaporating dish. The residue obtained was weighed and kept closed in a dessicator for further studies.

**Extraction with chloroform**

Extraction of *C. paniculatum* Linn. leaves marc with chloroform was carried out by hot continuous extraction method using soxhlet apparatus. 222g of marc was packed in the extractor. 300 ml of the solvent was taken in the round bottom flask and extraction was continued for 12 hours. The extract obtained was collected by gentle heating. The concentrated extract was then weighed and stored in the dessicator for further studies.

**Extraction with ethyl acetate**

Extraction of C. *paniculatum* Linn. leaves marc with ethyl acetate was carried out by hot continuous extraction method using soxhlet apparatus. 204g of marc was packed in the extractor. 300 ml of the solvent was taken in the round bottom flask and extraction was continued for 10 hours. The extract obtained was collected by gentle heating. The concentrated extract was then weighed and stored in the desiccators for further studies.

**Extraction with ethanol**

Extraction of *C. paniculatum* Linn. leaves marc with ethanol was carried out by hot continuous extraction method using soxhlet apparatus. 162g of marc was packed in the extractor. 300 ml of the solvent was taken in the round bottom flask and extraction was continued for 12 hours. The extract obtained was collected by gentle heating. The concentrated extract was then weighed and stored in the dessicator for further studies.

**Extraction with water**

Aqueous extraction was carried out with the remaining marc by reflux method. 100 g of marc was packed in a round bottom flask and refluxed for 2 hours using a reflux condenser. The extract obtained was then concentrated to dry residue by heating, weighed and used for further studies.

The percentage yields of the extracts were calculated and tabulated in the table:1.

## MATERIALS AND METHODS

The plant extracts were assessed for the existence of the phytoconstituents by using the following standard methods.[9,12]

### 1. Chemical tests for Alkaloids

**a) Mayer's test**

Few ml of the extracts was treated with two drops of Mayer's reagent (potassium mercuric iodide) along the sides of the test tube. The formation of white or creamy precipitate indicated the presence of alkaloids.

**b) Dragendorff's test**

Few ml of the extract was treated with 1 or 2 ml of Dragendorff's reagent (potassium bismuth iodide). The formation of reddish orange precipitate indicated the presence of alkaloids.

**c) Wagner's test**

Few ml of the extract was treated with Wagner's reagent (Iodine in potassium iodide). The formation of reddish brown precipitate indicated the presence of alkaloid.

**d) Hager's test**

Few ml of the extract was treated with 1 or 2 ml of Hager's reagent (Picric acid solution). The formation of yellow precipitate indicated the presence of alkaloids.

### 2. Chemical tests for Amino Acids and Proteins

**a) Ninhydrin test**

2ml of the extract was treated with 3ml of Ninhydrin reagent. The formation of purple colour indicated the presence of amino acids.

**b) Millon's test**

2ml of the extract was treated with few drops of. Millon's reagent. Brown colour indicated the presence of amino acids

**3. Chemical tests for Carbohydrates**

**a) Molisch's test**

Few ml of the extract was treated with two drops of alcoholic solution of α-naphthol. The mixture is shaken well and 1 ml of concentrated sulphuric acid is added solely along the sides of the test tube and allowed to stand. A violet ring at the junction indicated the presence of carbohydrates.

**b) Fehling's test**

1ml of the extract was treated with 1 ml each of Fehling's solution A and B and heated on a water bath for few minutes. The formation of red precipitate of cuprous oxide indicated the presence of carbohydrates.

**c) Barfoed's test**

1ml of the extracts was treated with 3ml Barfoed's reagent and heated on a water bath. The formation of red colour indicated the presence of carbohydrates.

**4. Chemical tests for Flavonoids**

**a) Shinoda test**

Small quantities of the extract was dissolved in alcohol, and few fragments of magnesium turnings and conc hydrochloric acid (drop wise) were added. Formation of pink or crimson-red colour indicated the presence of flavonoids.

**b) Concentrated sulphuric acid test**

2ml of the extract was treated with conc. sulphuric acidgb; the formation of orange colour indicated the presence of flavonoids.

**5. Chemical tests for Glycosides**

**a) Legal test**

2ml of the extract was dissolved in 1ml of pyridine and sodium nitroprusside solution was added. The mixture was made alkaline with Sodium hydroxide solution, the formation of pink or red colour indicated the presence of glycosides.

**b) Baljet test**

2ml of the extract was treated with 1ml of sodium picrate solution. The formation of yellow to orange colour indicated the presence of glycosides.

**6. Chemical tests for Saponins**

**a) Froth test**

About 10ml of the extract was mixed with 5 ml of water and shake well and observed for the formation of froth, which lasts for a long time.

**7. Chemical tests for sterols**

**a) Liebermann-Burchard test**

1ml of the extract was treated with 0.2ml con. sulphuric acid, 4ml of acetic anhydride and 20ml of chloroform. The formation of green colour indicated the presence of sterols.

**b) Salkowsky test**

2ml of the extract was treated with 5ml of con. sulphuric acid. The formation of red to purple colour indicated the presence of sterols.

**8. Chemical tests for Tannins and Phenolics**

**a) Ferric chloride test**

A little of the extract was dissolved in 1ml of water and few drops of 10% Ferric chloride solution was added. The formation of blue or green colour indicated the presence of tannins and phenolics.

**b) Lead acetate test**

1ml of the extract was treated with 10% lead acetate solution. The formation of white precipitate indicated the presence of tannins and phenolics.

**9. Chemical tests for terpenoids**

About 1ml of the extract was treated with 2ml of chloroform and 3ml of conc. sulphuric acid was carefully added to form a layer. The formation of reddish brown coloration on the interface indicated the presence of terpenoids.

**Invitro antioxidant activity**

Method used for antioxidant activity was β-carotene bleaching (BCB) method and Nitric oxide radical scavenging assay.

**β-carotene bleaching (BCB) method**

**Equipments**

Balance: Shimadzu digital electronic balance.

UV spectra: Shimadzu UV-1601 UV-VIS spectrophotometer.

The antioxidant activity of the extract was assayed based on the β-carotene bleaching (BCB) method.[13] 1 ml of beta carotene solution (0.2mg/ml chloroform) was added to 0.02 ml of linoleic acid and 0.2 ml of tween 20. After evaporation of chloroform it was diluted to 50 ml with distilled water. 10 mg of each extract was dissolved in 1ml of ethanol and from this 0.2 ml containing 2mg was used for the assay. 10 mg of propyl gallate was dissolved in 1ml methanol and from this 0.2 ml was used for the assay. A control containing 0.2 ml of ethanol was used for the assay.

0.2 ml of standard, sample and control were placed in different test tubes. To each test tube, 5 ml of beta carotene emulsion was added. The tubes were gentle shaken and placed at 45°C in a water bath for 60 minutes. The absorbance of the standard, sample and control was measured at 470 nm using UV/VISIBLE spectrophotometer against a blank, consisting of an emulsion without beta carotene. The measurement was carried out at initial time (t = 0) and successively at 30 and 60 minutes. All samples were assayed in triplicate and averaged. The antioxidant activity was measured using the equation,

AA = 100(DRC -DRS)/DRC, where AA = antioxidant activity; DRC = degradation rate of the control = [ln(a/b)/60]; DRS = degradation rate in presence of the sample = [ln(a/b)/60]; a = absorbance at time 0; b = absorbance at 60 min.

**Nitric oxide radical scavenging assay**

Nitric oxide radical scavenging assay was estimated using Griess Illosvory reaction.[14] 10 mg of sample extracts of *C. paniculatum* Linn. was weighed accurately and dissolved in phosphate buffer saline and made up to 10 ml with the same to obtain 1mg/ml solution. From the above solution, 0.05, 0.1, 0.15, 0.2ml were pipetted out and made up to 0.5 ml with phosphate buffer saline to get 50, 100, 150, 200µg/ml solutions. Here quercetin was used as the standard. The standard solutions were prepared by weighing 10mg of the quercetin and proceeding in the same manner as that of the sample to get 50, 100, 150, 200 µg/ml for comparison.

The reaction mixture (3ml) containing sodium nitroprusside (2ml), phosphate buffer saline (0.5ml) and standard solution (0.5ml) was incubated at 25°C for 2.5 hours. After incubation, 0.5ml of the reaction mixture containing nitrate was pipetted out and mixed with 1ml of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 minutes for completing diazotization. Then 1ml of 1-Naphthylamine was added, mixed and allowed to stand for 30 minutes at room temperature. A pink colour was formed in diffused light. A control was also prepared by the above solutions without standard. The absorbance of the solution was measured at 540nm against the corresponding blank solution. All samples were assayed in triplicate and averaged. The percentage inhibition was calculated for the samples and standard using the following equation,

$$\% \text{ Inhibition} = \frac{Ac - As}{Ac} \times 100$$

Ac is the absorbance of the control and As is the absorbance of sample/standard.

**Calculation of 50% Inhibitory Concentration ($IC_{50}$)**

The concentration (µg/ ml) of the various extracts required to scavenge 50% of the radicals was calculated in nitric oxide radical scavenging assay by using the percentage scavenging activities at four different concentrations of the extracts. The graphical representations and the calculated IC50 values are depicted in the results and discussions.

**Statistical analysis**

Statistical analysis Results of the research were tested for statistical significance by one-way ANOVA. Differences were considered statistically significant at the $P < 0.05$ level.

## RESULTS AND DISCUSSION

**Extractive values**

*Clerodendrum paniculatum* Linn. leaves were extracted with petroleum ether by simple maceration yielded 8.14 g of extract. The remaining marc extracted with chloroform by soxhlet extraction yielded 4.81g and the ethyl acetate extraction of the remaining marc by soxhlet extraction yielded 2.14g. The remaining marc was extracted with ethanol by soxhlet extraction and yielded 1.44g and the aqueous extraction of the remaining marc by reflux method yielded 0.5g. The percentage yield of the same were calculated and tabulated in the table below.

**Table 1: Extractive values.**

| Extracts | Percentage Yield (%w/w) |
|---|---|
| Petroleum ether extract | 1.16 |
| Chloroform extract | 2.17 |
| Ethyl acetate extract | 1.05 |
| Alcoholic extract | 0.89 |
| Aqueous extract | 0.5 |

**Preliminary phytochemical evaluation**

All five extracts were subjected for chemical tests for the identification of various phytoconstituents. The results of the chemical tests for each extract was recorded and tabulated below.

**Table 2: Phytoconstituents Present in Each Extract.**

| Phytoconstituents | PE | CE | EA | AE | AQE |
|---|---|---|---|---|---|
| **Alkaloids** | | | | | |
| Mayer's test | + | + | + | + | - |
| Wagner's test | + | + | + | + | - |
| Hager's test | + | + | + | + | - |
| Dragendroff's test | + | + | + | + | - |
| **Terpenoids** | | | | | |
| Isoprenoid test | ++ | + | - | - | - |
| **Sterols** | | | | | |
| Libermann burchard test | ++ | + | - | - | - |
| Salkowski's test | ++ | + | - | - | - |
| **Flavanones and Flavonoids** | | | | | |
| Aqueous sodium hydroxide test | - | + | ++ | + | - |
| Shinoda's test | - | + | ++ | + | - |
| **Phenolics and tannins** | | | | | |
| Ferric chloride test | - | + | ++ | + | - |
| Lead acetate test | - | + | ++ | + | - |
| **Carbohydrates** | | | | | |
| Molisch's test | - | - | - | + | + |
| Benedict test | - | - | - | + | + |
| Fehlings test | - | - | - | + | + |
| **Glycosides** | | | | | |
| Legal test | - | - | + | + | + |
| Baljet test | - | - | + | + | + |
| **Proteins and aminoacids** | | | | | |
| Millon's test | - | - | - | - | + |
| Biuret test | - | - | - | - | + |
| Ninhydrin test | - | - | - | - | + |
| **Saponins** | | | | | |
| Foam test/froth test | - | - | - | - | - |

**(++) Active constituents (high)**
**(+) Presence of active constituents**

**(-) Absence of active constituents**

The petroleum ether extract showed the presence of terpenoids, sterols and alkaloids. The chloroform extract showed the presence of phenolics, flavonoids, terpenoids, alkaloids and steroids. The ethyl acetate extract showed the presence of phenolics, flavonoids, alkaloids and glycosides. The alcoholic extract showed the presence of phenolics, flavonoids, alkaloids, carbohydrate and glycosides. The aqueous extract showed the presence of carbohydrate, glycosides and proteins.

**Invitro antioxidant activity**

**β-carotene bleaching (BCB) method**

The antioxidant activity of the extracts was evaluated by the beta carotene linoleic acid test systems. In the absence of antioxidants, the oxidation products (lipid hydroxides, conjugated dienes and volatile byproducts) of linoleic acid attack beta carotene, resulting in bleaching of its characteristic yellow colour in the ethanol solution. In the presence of extracts, oxidation products were scavenged and bleaching was prevented. The absorbance obtained for control, standard and samples at 0, 30, and 60$^{th}$ minute were recorded and the % antioxidant activity was calculated and tabulated below.

**Table 3: The Absorbance obtained for control, standard and samples at 0, 30, and 60$^{th}$ minute and the % Antioxidant activity by β-carotene bleaching (BCB) method.**

| Sample | Absorbance at 470 nm | | % antioxidant activity | | |
|---|---|---|---|---|---|
| | 0 min | 30 min | 60 min | 30 min | 60 min |
| Control | 0.144±0.0020 | 0.129±0.0031 | 0.120±0.0026 | | |
| Standard drug | 0.206±0.0012 | 0.203±0.0021 | 0..201±0.0028 | 80.4 | 79.2 |
| Petroleum ether extract | 0.530±0.0023 | 0.522±0.004 | 0.516±0.002 | 46.7 | 41.7 |
| Chloroform extract | 0.221±0.0023 | 0.215±0.0023 | 0.210±0.003 | 60.0 | 54.2 |
| Ethyl acetate extract | 0.354±0.002 | 0.350±0.003 | 0.346±0.0021 | 73.4 | 66.7 |
| Alcoholic extract | 0.488±0.0060 | 0.483±0.007 | 0.479±0.005 | 66.7 | 62.5 |
| Aqueous extract | 0.494±0.002 | 0.487±0.001 | 0.482±0.003 | 53.3 | 50.0 |

In the beta carotene bleaching method, it was found that the ethyl acetate extract of *C. paniculatum* Linn. has good free radical inhibition activity than the other extracts. Also, the chloroform and alcoholic extracts have comparable antioxidant activity with that of the standard.

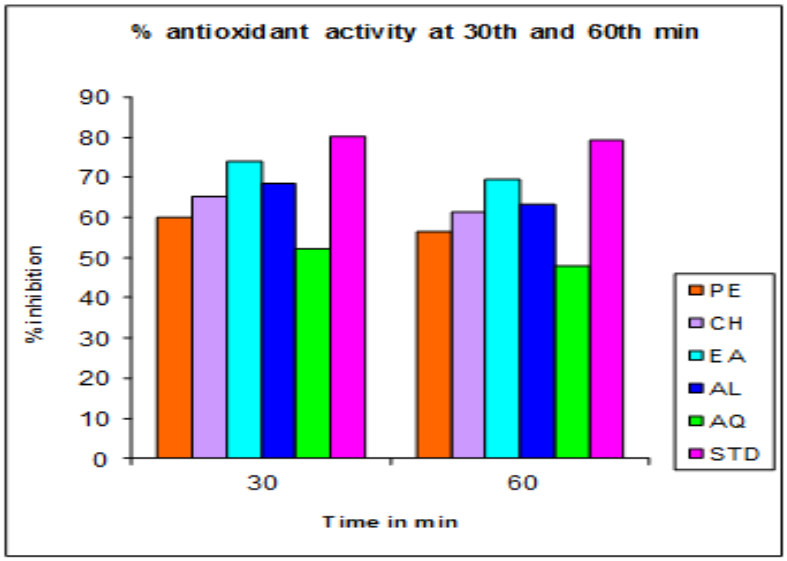


**Fig. 1: Comparative antioxidant study of extracts and the standard at 30$^{th}$ and 60$^{th}$ min by β-carotene bleaching (BCB) method.**

**Nitric oxide radical scavenging assay**

In nitric oxide radical scavenging assay, the inhibition of nitric oxide formation was calculated. The plant/ plant products with the property to counteract the effect of NO formation may be considered as very effective in preventing the ill effects of excessive NO generation *in vivo*. The reduction in the NO is relevant from the reduction in the absorbance of reaction mixture. The absorbance of reaction mixture in the presence of different concentrations of standards and samples were recorded. The % inhibition were calculated for the standards and samples and tabulated in the table below.

**Table 4: % inhibition by Nitric oxide radical scavenging assay.**

| Extracts | Concentrations | Absorbance(nm) | % inhibition(µg/ml) |
|---|---|---|---|
| Control | | 0.217 | |
| Petroleum Ether extract | 50 | 0.192±0.003 | 11.52 |
| | 100 | 0.153±0.001 | 29.49 |
| | 150 | 0.127±0.002 | 41.40 |
| | 200 | 0.100±0.002 | 53.91 |
| chloroform extract | 50 | 0.188±0.002 | 13.36 |
| | 100 | 0.145±0.002 | 33.18 |
| | 150 | 0.090±0.003 | 58.5 |
| | 200 | 0.064±0.004 | 70.5 |
| Ethyl acetate extract | | 0.175±0.003 | 19.35 |
| | | 0.130±0.003 | 40.09 |
| | | 0.075±0.004 | 65.43 |
| | | 0.038±0.006 | 82.48 |
| Alcoholic extract | 50 | 0.186±0.003 | 14.28 |
| | 100 | 0.140±0.002 | 35.4 |
| | 150 | 0.087±0.003 | 59.9 |

| | | | |
|---|---|---|---|
| | 200 | 0.058±0.004 | 73.27 |
| Aqueous extract | | 0.190±0.004 | 12.44 |
| | | 0.148±0.003 | 31.79 |
| | | 0.110±0.004 | 49.3 |
| | | 0.068±0.004 | 68.66 |
| Standard drug | | 0.171±0.005 | 21.19 |
| | | 0.125±0.005 | 42.39 |
| | | 0.068±0.006 | 68.66 |
| | | 0.022±0.006 | 89.86 |

$IC_{50}$ values were calculated for each extract of *C. paniculatum* Linn. leaves and standard from the graph. The values obtained are tabulated in table 5. The graphical representation of the results is depicted in figure. 2.

**Table 5: $IC_{50}$ value in (µg/ml) of different extracts.**

| Sl.No: | Extract | $IC_{50}$ value(µg/ml) |
|---|---|---|
| 1. | Petroleum ether extract | 185.0 |
| 2. | Chloroform extract | 133.8 |
| 3. | Ethyl acetate extract | 119.6 |
| 4. | Alcohol extract | 130.2 |
| 5. | Aqueous extract | 152.1 |
| 6. | Standard drug | 114.8 |

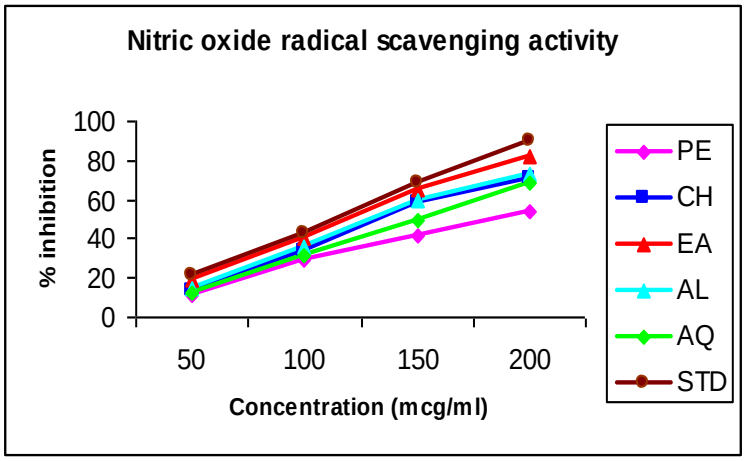


**Fig. 2: Comparative $IC_{50}$ for the extracts of *C.paniculatum* and standard drug.**

The extract showed potent scavenging activity of nitric oxide and the $IC_{50}$value of ethyl acetate extract is comparable to that of the standard.

## CONCLUSION

The phytochemical analysis showed that the *Clerodendrum paniculatum* plant extract contains a mixture of phytochemicals as reducing sugars, cardiac glycoside, phenolic compounds, flavonoids, and alkaloids. The β-carotene bleaching (BCB) method and Nitric

oxide radical scavenging assay indicated that the plant extract has potent antioxidant activity which can be an excellent option for biological and chemical analysis and can be further subjected for the isolation of the therapeutically active compounds. This leaf extract is a promising candidate that can be used as natural products based antioxidant.

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