

Antioxidant activity of essential oil extracted from *enicostemma littorale*Gayathri P K¹, Sathish Kumar K^{2*}¹Department of Biotechnology, Vel Tech High Tech Dr. Rangarajan Dr. Sakunthala Engineering College, Avadi, Chennai -600 062.²Department of Chemical Engineering, SSN College of Engineering, Kalavakkam – 603110.***Corresponding author: E-Mail: sathishkumark@ssn.edu.in****ABSTRACT**

The main aim of this study is to determine the antioxidant effect of the phenol isolated from the leaf of *Enicostemma littorale* (*E. littorale*). The phenols were extracted from the leaf of *E. littorale* using 5 different solvents (water, methanol, ethanol, acetone, petroleum ether). The concentrate was taken up in methanol and the antioxidant activity was determined using DPPH assay. Except the pet ether extract, all the extracts showed positive result on analyzing the phenol content using Folin-Ciocalteu's reagent. It was observed that the aqueous extract showed best antioxidant activity (>90%) when compared with the positive control BHT. Further investigations shall be concentrated on the antiproliferative activity of the phenol compound isolated from the leaves of *E. littorale*.

KEY WORDS: Phenol, Folin's reagent, DPPH assay, phytochemicals.**1. INTRODUCTION**

Vellarugu (*Enicostemma littorale*) is a glabrous herb with sessile lanceolate leaves which grows from 5 – 10 cm length and is found throughout India. The secondary metabolites from the leaves of *E. littorale* were found to possess hypoglycemic, hepatoprotective and hepatomodulatory properties and helps in reducing obesity. Medicinal compounds derived from these plants were considered to be very effective since these were less toxic, eco-friendly, palatable and free from side effects. *E. littorale* is a good source of iron, potassium, sodium, calcium, magnesium, silica, chloride, sulphate, phosphate and vitamins B and C.

According to the World Health Organization (WHO), herbal medicines serve the health needs of about 80% of the world's population, especially for millions of people in the vast rural areas of developing countries. India being a huge nation of natural resources has unexplored plant species with potent medicinal values. The age old ayurvedic therapy for the cure of many lives threatening disease like cancer, cardiac arrest, neurological disorders etc still proves to be the best medications. The methods of application vary from various locations with 80% of rural population relying as means of treating various diseases. But many of the procedures were not available for the public and still remain to be a great secret. It is the responsibility of the scientists to identify the unexplored treasure from ancient India.

The study on identification and extraction of phytochemicals has attracted many scientists in the recent days and the need to detect the new bioactive compounds has become the prime responsibility. These types of studies bring systemic solution to the scarcity of new additional resources for the pharmaceutical industry. Numerous investigations have shown that the phenolic compounds have potent antimicrobial, anti-inflammatory and antioxidant activity because of the presence of functional hydroxyl group in the aromatic ring. Phenolic compounds can be prepared synthetically and it can also be produced by extraction from plants and microbes. The world is trying to go organic and hence extraction from the plants holds to be stable and safe for the medicinal use of the human beings. The current study deals with the extraction of one such phenolic compound from the essential oil of *E. littorale* and determines its antioxidant property. Based on the results obtained in this study future research shall be concentrated on converting this phenolic compound for the anti-proliferative therapy.

2. MATERIALS AND METHODS

2.1. Materials required: Ethanol, Petroleum ether, chloroform, Acetone, distilled water, Na₂CO₃, Folic Ciocalteu reagent, BHT (butylated hydroxyl toluene), DPPH (2, 2-dyphenyl-1-picrylhydrazyl). All the chemicals used were of analytical grade and were procured from Sisco chemicals.

2.2. Collection and identification of plants: The *E. littorale* plant was collected from Siddha Hospital at Chennai and was identified by experts at Vel Tech High Tech Dr. Rangarajan Dr. Sakunthala Engineering College. The collected leaves of the plant were cleansed; shade dried for 10 days and was pulverized by mortar and pestle. The powdered leaves were packed in a dry air tight sterilized bottle and were used for further studies.

2.3. Extraction of essential oil: The essential oil from the powdered plant leaf was extracted by using 5 different solvents such as petroleum ether, chloroform, acetone, ethanol and water by heating at 45°C for min. The extract was filtered by Whattman No.1 filter paper and the filtrate was stored in a brown bottle.

2.4. Qualitative analysis for phenolic compound: The qualitative analysis for the presence of phenol in each extracts was determined by the methods described elsewhere. Briefly, 1 ml of leaf extract was mixed with 1 ml of 7.5 % sodium carbonate and 1 ml of Folin-Ciocalteu's reagent. The formation of blue or green color indicates the presence of phenol.

2.5. DPPH assay: DPPH is the standard assay to test for the antioxidant capacity of any compound. The leaf extracts were mixed with 0.1% methanolic DPPH and was incubated for 30 min in dark. The purple color indicates the absence of antioxidant activity and yellow color indicates that the compound has antioxidant potential. The percentage of DPPH radical inhibition was calculated from the formula given below using the absorbance value obtained by UV double beam spectrometer at 517 nm.

$$\% \text{ inhibition} = 100 (\text{Abs of control} - \text{Abs of sample}) / \text{Abs of control}$$

3. RESULTS AND DISCUSSIONS

3.1. Extraction of bioactive compounds: The five different extracts were shown in fig 1. The main objective of the study was to extract phenolic compound and to investigate for its antioxidant potential. High concentrations of phenols can be extracted from a plant sample by using polar solvents and the same was followed for the study to be conducted. The degree of polarity of the solvents used shall be given as Water>Ethanol>Acetone> Chloroform> Petroleum ether. It was expected that the concentration of phenolic content extracted by these solvent shall be equivalent to the same order shown above.

3.2. Qualitative analysis for phenolic compound: The preliminary screening of the extracts for the presence of phenol was performed and it was observed that except petroleum ether all the other extracts showed positive results for the presence of phenol. On adding Folin's reagent the extracts indicated blue, green or blue-green color (fig 2). The clear blue color indicates the strongest presence of phenolic compound and it was observed in the aqueous extract. The results in the fig 2 showed that highly polar solvent showed strongest positive results for the presence of phenol which was in good agreement with the discussion given above. Phenols have the ability to efficiently scavenge free radicals due to its hydroxyl group and the presence of phenolic compound may contribute for the antioxidant activity of the plant extracts which was confirmed by numerous investigations earlier.

3.3. DPPH assay: Free radical and reactive oxygen species are inducers of cellular and tissue pathogenesis leading to many human diseases. Many plant species with antioxidant activities act as protective agents against cancer, diabetes and aging process. The qualitative results of DPPH assay against the leaf extracts are depicted in fig.3 and it was observed that the ethanol, acetone and aqueous extract showed positive results (yellow color) while the other extracts showed negative results (purple color).

The change of color from purple to yellow is due to the quenching of DPPH free radical by the antioxidant molecule (phenol) in the leaf extracts. On the action of antioxidant molecule, the DPPH is converted into 2, 2-diphenyl-1-hydrazine (a bleached compound) which results in the decreased absorbance at 517 nm. The antioxidant activity of the leaf extracts were expressed as % of inhibition which is shown in fig 4. BHT was used as a positive control and plain DPPH solution without the extract was taken as the negative control. Among the five extracts, ethanol extract showed maximum antioxidant activity followed by water and acetone extract after 30 min incubation time. The petroleum ether and chloroform extract showed minimum antioxidant activity (<30%). The highest antioxidant activity depicted the maximum capacity to neutralize DPPH. On comparing fig 2 and 4, it was observed that the extracts which showed positive results for phenolic compound in qualitative analysis showed highest antioxidant potential.

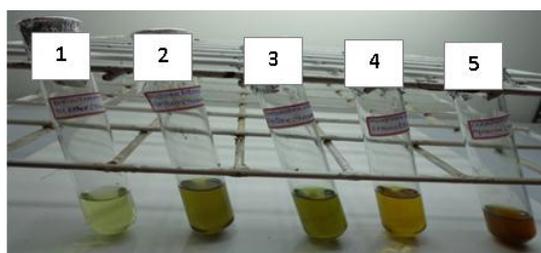


Figure.1. Extraction of phyto-constituents using 5 different solvents, 1.Petroleum ether, 2.Chloroform, 3.Acetone, 4.Ethanol, 5.Aqueous

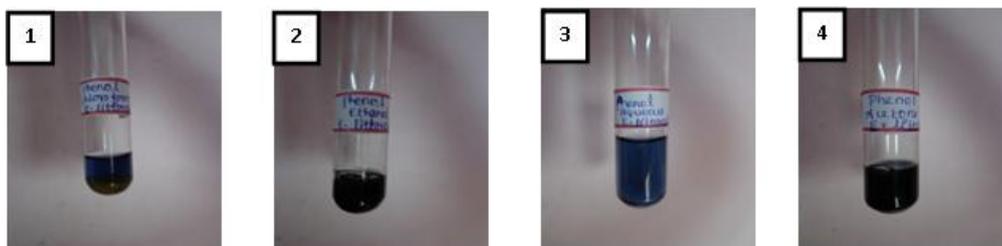


Figure.2. Positive results of qualitative analysis for phenolic compound, 1.Blue Green color of chloroform extract, 2.Green color of Ethanol extract, 3.Blue color of Aqueous extract, 4.Green color of Acetone extract

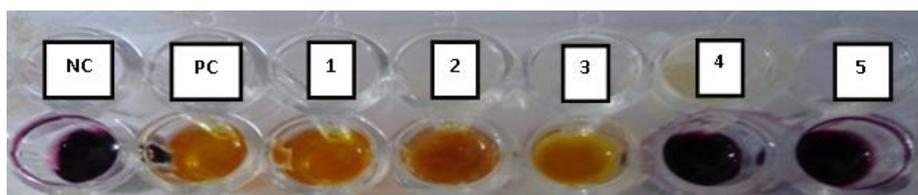


Figure.3. Qualitative DPPH assay for the leaf extracts, NC – Negative control, PC – Positive control (BHT), 1.ethanol extract (positive), 2.aqueous extract (positive), 3.acetone extract (positive), 4.chloroform extract (negative), 5.petroleum ether extract (negative)

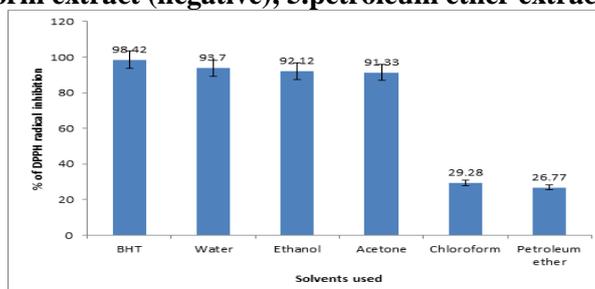


Figure.4. Anti-oxidant activity of the five leaf extracts shown as % of DPPH radical inhibition

4. CONCLUSION

From the results obtained by the study it shall be suggested that the aqueous extract of the *E. littorale* leaves showed potent antioxidant activity followed by ethanol and acetone extract. It shall also be concluded that the efficiency of antioxidant activity is dependent on the concentration of the phenolic content in the extract. The polarity of the solvent used also played a major role in the extraction of phenolic compound from the plant. Further studies shall be directed towards determining the anti-proliferative activity of the extract against various cancer cell lines and to obtain a valuable pharmaceutical product for the societal usage.

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