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Evaluation of In-Vitro Antioxidant Activity of Two Anti-Arthritic Plants by DPPH° Method

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Abstract:- The antioxidant properties of two anti-arthritic plants – Strobilanthes ciliatus and Canophylllum inophyllum was quantified by DPPH° method. These plants are proved to be rich in phytochemcials like phenols, flavonoids, turpenoids, etc. Roots of Strobilanthes ciliatus and leaves of Canophylllum inophyllum was taken for the study. The % inhibition of DPPH° was found and IC_{50} value was calculated. From the results obtained it is found that a good correlation exists between phenolic phyto constituents & antioxidant property by DPPH° scavenging method.

Keywords:- *Strobilanthes ciliatus, Calophyllum inophyllum, DPPH° scavenging method, IC_{50} value.*

I. INTRODUCTION

Since ancient time, herbal medications find applications for relief of ailments (Maqsood et al., 2010). As crude concentrates or their active constituents natural antioxidants are very adequate to inhibit the damaging processes formed by oxidative stress. Medicines of natural origin are much safer than synthetic drugs. (Vongtau et al., 2005). Antioxidants maintain or inactivate free radicals, often before they attack targets in biological cells. The role of free radical reactions in disease pathology is well known and is found to be involved in many acute and chronic ailments in human beings, such as diabetes, atherosclerosis, aging, immunosuppression and neurodegeneration.

The antiarthritic properties in plants are greatly influence by the antioxidant activity. There are a number of plants used for the treatment of arthritis in traditional medicine. In the present study the roots of Strobilanthes ciliates and leaves of Calophyllum inophyllum was selected because both these plants are traditionally used for the treatment of arthritis. The extracts of both plants on qualitative phytochemical investigation was found to be rich in Phenolics, Flavonoids, Glycosides, Terpenoids, Sterols and saponins. Determination of total phenol content and total flavonoid content of the extracts revealed high phenolic and flavonoid contents in both the plants. Therefore the present study was aimed to analyse the extracts for antioxidant activity by DPPH° method.

II. MATERIALS AND METHOD

A. Plant collection:

The roots of *Strobilanthes ciliates* were collected from Ranny, Pathanamthitta, Kerala. The leaves of *Calophyllum inophyllum* were collected from Changanacherry, Kottayam, Kerala. Both the plant specimens were authenticated by Dr. Vinodkumar T.G., St. Thomas College, Ranny.

B. Extraction of plant material:

The roots of Strobilanthes ciliatus were thoroughly washed, shade dried, powdered (1kg) and was subjected to sequential extraction separately with pet. ether (60-80 ℃), $CHCl_3$, ethyl acetate, methanol and water in a Soxhlet extractor. The extracts were concentrated to dryness. The obtained extracts were kept in desiccators to remove moisture and stored properly until used.

The leaves of Calophyllum inophyllum were thoroughly washed, shade dried and roughly powdered (750 gm). The powder was macerated with methanol in a round bottom flask for 7 days. To ensure the efficiency of the extraction the contents of flask were stirred intermittently. The essence was filtered and the filtrate was evaporated. The procured extract was kept in desiccators to abolish moisture and stored properly until used.

C. In-vitro Antioxidant Activity by Free radical scavenging activity on DPPH° .

The DPPH° radical scavenging activity of ethyl acetate, methanol and aqueous extracts of *Strobilanthes ciliatus* roots and methanolic extract of *Calophyllum inophyllum* leaves was measured. (Blios, 1958). Concentration of concentrate to decrease the initial concentration of DPPH° by 50% (IC_{50}) was calculated.

3ml of 0.004% DPPH° solution in methanol was mixed with 2ml of plant extract solutions of varying concentrations (12.5, 25, 50, 100, 200, 400 µg/ml). Analogues blank sample were prepared and Ascorbic acid was used as reference standard. Mixer of 3ml methanol and 1ml DPPH solution was used as control. The reaction was carried out in triplicate and the decrease in optical density was measured at 517nm after 30 minutes in dark using UV-Vis spectrophotometer. The inhibition % which is the radical scavenging capacity was calculated using the following formula.

Inhibition % = Ac-As/Ac×100

Where Ac = absorbance of the control

As = absorbance of the sample or standard.

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III. RESULTS

In the presence of an antioxidant which can donate an electron to DPPH° , the purple colour of typical free DPPH° radical decays and the change in absorbency at 517nm is measured. The DPPH° is a stable radical with λ_{max} of 517 nm which undergo scavenging by antioxidant (Lu and Yeap, 2001). It is used widely to test the potentiality of compounds as free-radical scavengers or hydrogen donors and to analyse the antioxidative activity of plant concentrate. % inhibition was plotted against concn. and from it IC_{50} was calculated. The scavenging abilities of various solvent concentrate of *Strobilanthes ciliatus* and *Calophyllum inophyllum* were expressed as IC_{50} values as shown in Table:1 and Figure:1-5. A lower IC_{50} value indicated higher antioxidant activity.

Conc. of extracts (µg/ml)	Strobilanthes ciliatus				Calophyllum inophyllum
	Ascorbic acid	Ethyl acetate extract	Methanolic extract	Aqueous extract	Methanolic extract
12.5	31.21±0.02	18.08±0.21	35.15±0.38	14.51±0.41	24.02±0.36
25	47.04±0.08	27.23±0.24	44.04±0.09	23.78±0.03	37.54±0.05
50	58.25±0.21	39.54±0.06	50.14±0.17	38.35±0.25	62.35±0.21
100	63.01±0.13	58.14±0.34	60.47±0.35	49.24±0.42	69.52±0.42
200	70.32±0.04	74.21±0.26	69.11±0.11	63.84±0.05	73.54±0.34
400	91.18±0.22	84.24±0.01	84.21±0.02	92.34±0.11	86.55±0.16
IC_{50} value (µg/ml)	51.01	129.73	68.39	147.43	62.39

Table 1. DPPH° scavenging activities of ethyl acetate, methanolic and aqueous extract of Strobilanthes ciliates and methanolic extract of Calophyllum inophyllum (values represent means ±S.D, n=3)

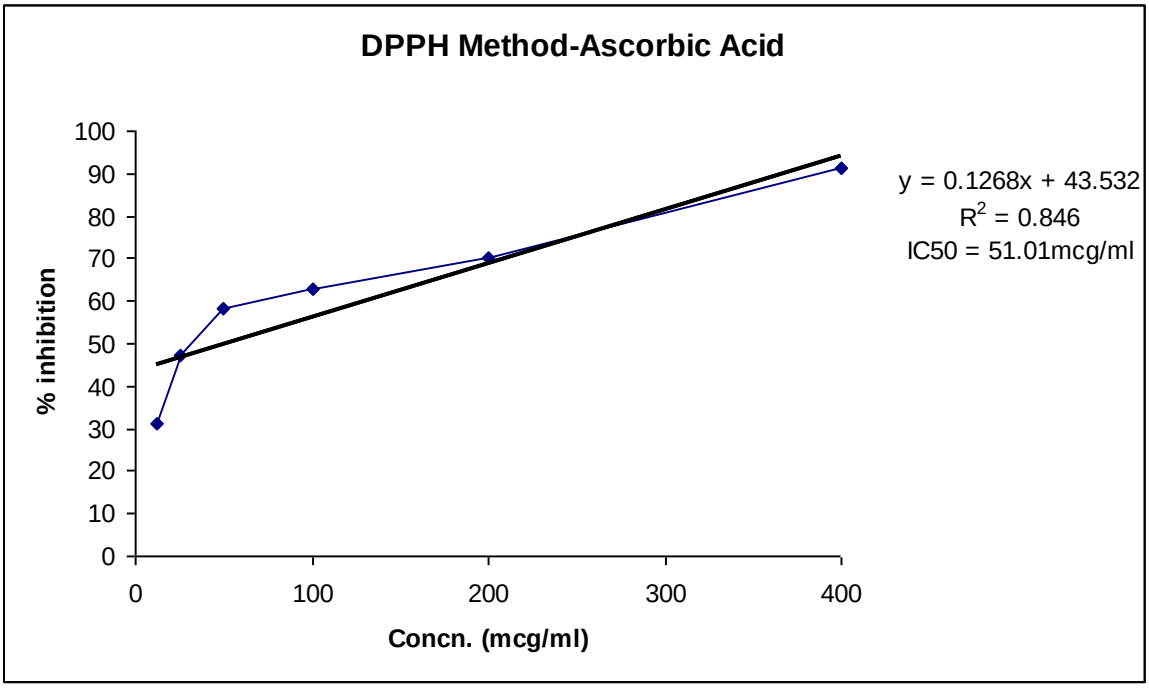

Fig 1:- DPPH° free-radical scavenging activity in different concentrations of Ascorbic acid

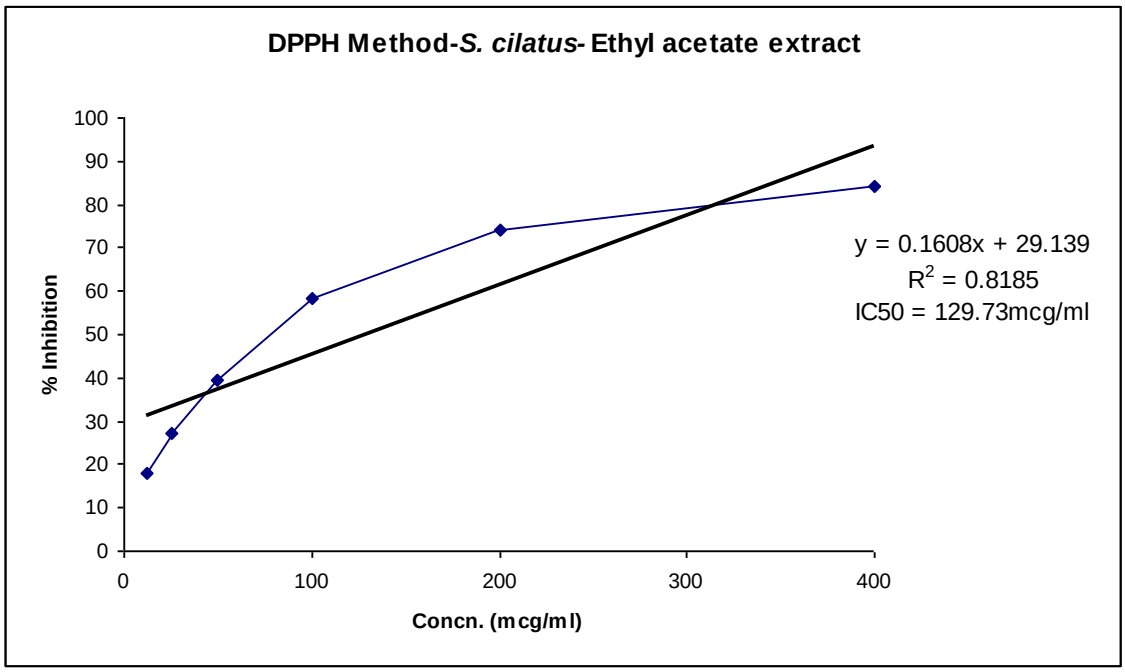

Fig 2:- DPPH° free-radical scavenging activity in different concentrations of ethyl acetate extract of Strobilanthes ciliatus roots

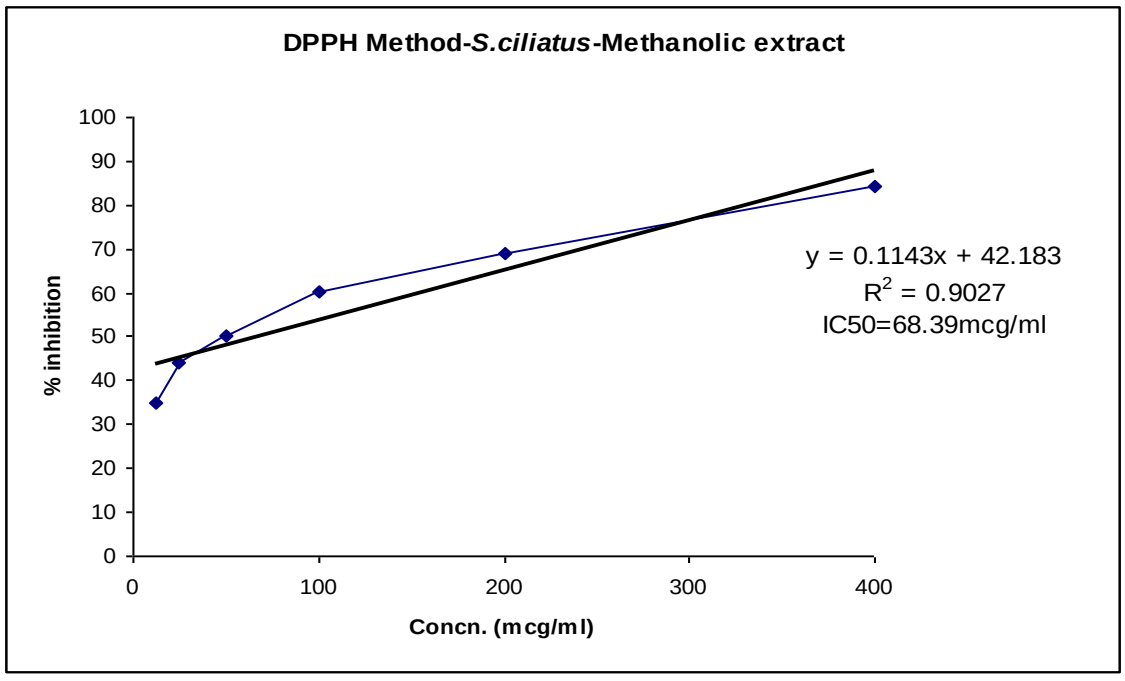

Fig 3:- DPPH° free-radical scavenging activity in different concentrations of methanolic extract of Strobilanthes ciliatus roots

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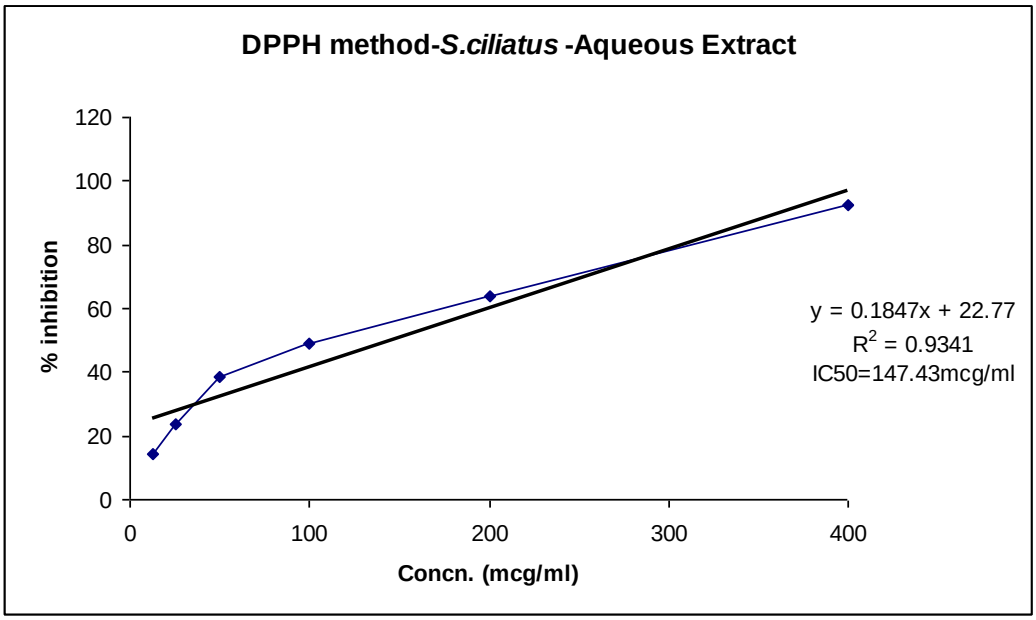

Fig 4:- DPPH° free-radical scavenging activity in different concentrations of aqueous extract of Strobilanthes ciliatus roots

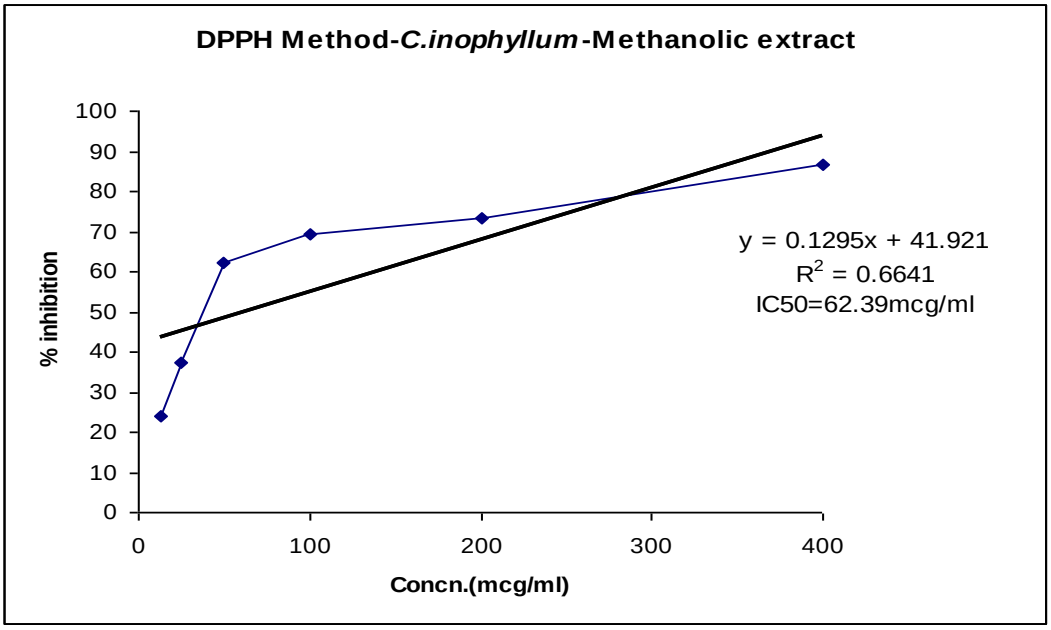

Fig 5:- DPPH° free-radical scavenging activity in different concentrations of methanolic extract of Calophyllum inophyllum leaves

IV. DISCUSSION AND CONCLUSION

Recent reports have shown that majority of polyphenolic constituents derived from plants are more effective antioxidants *in-vitro* than vitamins E or C, and thus might contribute significantly to the protective effects *in-vivo*. Many *in-vitro* methods have been used to find the antioxidant activity *in* order to permit fast screening of substances. It can be suggested that mostly those compounds which show low antioxidant activity by in- *vitro* methods, will possibly show little activity by *in-vivo* methods. Free radicals have a very significant part to play in a wide variety of pathological conditions. Antioxidants produce their action either by scavenging the ROS or by securing the antioxidant defence mechanism. Thus antioxidants act against free radicals and

protect us from various diseases. (Umamaheswari and Chatterjee, 2008).

The ability of a compound to donate the electron can be measured by bleaching of purple coloured 2,2′-diphenyl-1-picrylhydrazyl radical (Nunes *et al*., 2012). In this method DPPH° radical is scavenged by the addition of a species or antioxidant that decolourizes the DPPH° solution. The degree of colour change of DPPH° solution is a measure of concentration and potency of the antioxidants. A low value of optical density of the reaction mixture indicates tht the compound under test is having good DPPH° radical scavenging activity. In the present study among all the fractions tested, methanolic extract of both *Strobilanthes ciliates* and *Calophyllum inophyllum* displayed significantly higher % inhibition and positively corresponded with tot. phenolic content. Results of present study recommend that the plant concentrate contain phytochemical constituents which donate hydrogen to a free radical to scavenge the potential damage.

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