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"In Vitro Antioxidant and Phytochemical Analysis of *Acalypha alnifolia Klein Ex Willd".*

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Abstract: Herbs have been utilized to treat acute and chronic disorders for thousands of years. Natural products, due to their easy availability, low cost and lack of side effects, make themselves attractive candidates for drug research. One such indigenous medicinal plant is *Acalypha alnifolia* Klein ex Willd, belonging to the family Euphobiaceae was selected. There is no systematic work that has been undertaken on this plant. The objective of the present study was to examine the antioxidant potential and phytochemical analysis of *A.alnifolia* leaves. The results of Phytochemicals analysis showed total phenols, flavonoids and protein were positive in leaf extracts. The free radical scavenging effect and reducing power were analyzed in methanol and aqueous extracts of *A.alnifolia* leaves. The results highlighted both extract exhibited better antioxidant activity. This study is the first report of antioxidant and phytochemical analysis of *A.alnifolia.* Based on findings, the leaf extracts may be used as an effective antioxidant agents to combat various ailments caused by the free radicals.

Key Words: *Acalypha alnifolia* , antioxidant, phytochemical, crude extracts.

I. Introduction

Oxidation and the production of free radicals are an integral part of human metabolism. Oxygen free radicals or more generally reactive oxygen species (ROS), as well as reactive nitrogen species (RNS) are products of normal cellular metabolism. ROS and RNS are well recognized for playing a dual role as both deleterious and beneficial species, since they can be either harmful or beneficial to living system (Valko *et al.*, 2006).

Reactive oxygen and nitrogen species can attack various substrates in the body including lipids, nucleic acids and protein. Oxidation of any of these substrates can theoretically contribute chronic diseases such as cancer, cardiovascular diseases and age related macular degeneration and to aging (Seitz and Stickel, 2006).

Production of reactive oxygen species (ROS) and defense system against them are balanced well in the living body. This balance is very much important for the maintenance of physiological condition. The collapse of the balance due to pathogenic infection and inflammation will induce the production of ROS and results in ROS toxicity. To control this toxicity the endogenous defense system start's its action against those pathogens to control ROS toxicity indirectly (Nishikawa,Inoue.,2008).

In addition to the endogenous defenses, consumption of dietary antioxidants could be an important aspect of body's defense mechanism to protect against free radicals, such as superoxide anion radicals, the hydroxyl radicals and other ROS, and also many antioxidants are being identified as anticarcinogens (Willet, 1994). Many antioxidant substances derived from dietary or medicinal plants are known to be effective and versatile chemo preventive and antitumoral agents in a number of experimental models of carcinogenesis (Borrelli *et al.*, 2004).

Many medicinal plants contain large amounts of antioxidants such as polyphenols, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Many of these phytochemicals possess significant antioxidant capacities that are associated with lower occurrence and lower mortality rates of several human diseases (Anderson et al., 2001).

It has been reported that there is an inverse relationship between the antioxidative status occurrence of human diseases (Rice-Evans, Sampson, Bramley, & Holloway, 1997). In addition, antioxidant compounds which are responsible for such antioxidants activity, could be isolated and then used as antioxidants for the prevention and treatment of free radical-related disorders (Middleton, Kandaswami, & Theoharides, 2000; Packer, Rimbach, & Virgili, 1999).

The medicinal plant *Acalypha alnifolia* belonging to the family Euphorbiaceae was selected for screening the antioxidant and phytochemical properties. The purpose of this study was to evaluate medicinal plants for the antioxidant activity. This evaluation is related to the total phenolic content and antioxidant activity to find out new potential sources of natural antioxidants. However, no systematic work has been under taken on analyzing the extracts of *A.alnifolia*. Based on the above informations this study was aimed at analyzing the leaf extracts of *A. alnifolia* on antioxidant and phytochemical nature under in vitro condition.

II. Material And Methods

Plant collection and identification

Fresh and healthy leaves of *A. alnifolia*, were collected randomly from the region of Yercaud and Kolli hills, South India. The nomenclature of collected plant materials was identified and authenticated by Botanical survey of India, southern circle, India [No:BSI/SRC/5/23/2010-11/Tech-1506]. The herbarium specimen was deposited on the Department of Biotechnology, Periyar University, Salem.

Extraction of plant materials

The collected leaves were shade-dried and coarsely powdered by using mixer grinder. These coarse powders (25 g) were taken for successive extraction in various solvents such as methanol, chloroform, ethyl acetate and petroleum ether (each 250 ml) by using Soxhlet apparatus. The collected extracts were vacuum dried and stored for further investigations. Simultaneously, aqueous was used for extraction of plant powder and the extractants were stored. The DMSO (Dimethyl sufloxide) is act as dissolved agents for these extracts.

III. Antioxidant Assay

Reducing power assay

The reducing power assay was carried out according to the method described by Yen et al.,(1995). Sample in 1ml distilled water was mixed with 2.5ml of 0.2M phosphate buffer and 2.5 ml of potassium ferricyanide. The mixture was incubated at 50ºC for 20 minutes. 2.5 ml of 10% TCA was added and centrifuged for 10 minutes at 1000g. To 2.5ml of the upper layer, 2.5 ml of distilled water and 0.5 ml of 0.1% $FeCl_3$ was added and the absorbance was measured at 700 nm in a spectrophotometer. Higher absorbance of the reaction mixture indicated greater reductive potential.

DPPH radical scavenging activity

The scavenging of DPPH radical was carried out according to the method described by Hsu et al (2003). Aliquots of 1 ml of the extract and 5 ml of freshly prepared 0.1mM DPPH methanolic solution were thoroughly mixed and kept for 50 minutes in dark. The absorbance of the reaction mixture at 517nm was read in a spectrophotometer. Methanol (1ml) replacing the extract serves as positive control. Methanol was used as blank. The percentage of free radical scavenging effect was calculated as follows

Scavenging effect (%) = [(A517control – A517test)/A517control] X 100

Where A517control= Absorbance of the control at 517nm

A517test = Absorbance of the test at 517nm.

Phytochemical Screening

The aqueous and methanolic extracts of leaves of *A.alnifolia*, were analyzed for the qualitative determination of phytochemical constituents as described by Harborne (1973), Trease and Evans (1989) and Sofowora (1993).

Quantitatie Determination Of Chemical Constituency

Preparation of fat free Sample: 2g of the sample were defatted with 100ml of diethyl ether using a soxhlet apparatus for 2 hrs.

IV. Method Of Estiation Of Terpenoids And Fixed Oil

AOAC- Official Methods of Analysis(1999).

Estimation Of Total Phenolics

The phenolic content in the plant material was estimated by the method of Okwu, (2005). For the extraction of the phenolic component, the fat free sample was boiled with 50 ml of ether for 15 minutes. To this, 5.0 ml of the extract, 10.0 ml of distilled water, 2.0 ml of ammonium hydroxide and 5.0 ml of concentrated amyl alcohol were added. The sample was left to react for 30 minutes for color development. The absorbance of the solution was read using a spectrophotometer (at 760 nm) wavelength. The results were expressed as mg of phenol/ gm of dried sample.

Estimation Of Total Flavonoid Content

The total flavonoid content in the sample was estimated by the method of Chang *et al.*, (2002). The extract prepared for the estimation of total phenolics was used as sample for this assay. 0.25 ml of the sample was diluted to 1.25 ml with distilled water. 75 µl of 5 % sodium nitrite was added and, add 0.15 ml of aluminium chloride solution was added. 0.5 ml of 0.1M NaOH was added after 5 minutes and made up to 2.5 ml with distilled water. The solution was mixed well and the absorbance was read (at 510 nm) in comparison with standard quercetin at 5-25 µg concentration. The results are expressed as mg of flavonoids as quercetin equivalent/ gm of dried sample.

V. Results

Result Of Antioxidant Assay

Reducing Power Assay

The leaf aqueous extract was very potent when compare to other form of extracts i.e. the leaf aqueous extract reduces the ferric cyanide to ferrous cyanide. The aqueous extract of the leaf shows the higher reductive activity than the other solvent extracts.

Reducing Power Assay

Sample	Absorbance at 770nm			
	Aqueous	Chloroform	Pet.ether	Methanol
Leaf extract	0.58035	0.05405	0.03155	0.4753

DPPH radical scavenging activity

DPPH Spectrophotometric Assay

The quantitative scavenging activities of various solvents extract of leaves were tested against DPPH by quantitative spectrophotometric assay. DPPH a stable free radical with a characteristic absorption at 517 nm was used to study the radical scavenging effects of extracts. The concentration of DPPH was highly decreased in the aqueous extracts of the leaves.

DPPH radical scavenging activity

Sample	% of Scavenging at 570nm			
	Aqueous	Chloroform	Pet.ether	Methanol
Leaf extract	91.565	52.555	48.34	88.760

Qualitative phytochemical analysis of leaf extracts of A.alnifolia

Sl/No	**Test**	**Methanolic Extract**	**Aqueous extract**
1	Test for carbohydrates a. Molisch's test	 +	 +
2	Test for Glycosides a. Keller-Killiani test	 -	 +
3	Test for Saponins a. Foam test	+	-
4	Test for Alkaloids a. Mayer's test b. b. Dragendrodroff's test	 + +	 + +
5	Test for Flavonoids a. Alkaline reagent test	 +	 +
6	Test for Phenolics and Tannins a. Ferric chloride test b. Test for Tannins	 + +	 + +
7	Test for Phytosterols and Triterpenoids a. Leiberman-Bucharat test b. Salkowaski test	 + +	 + -
8	Test for fixed oils and fats a. Oily spot test	 -	 -

(+) Present, (-) Absent

Quantitative phytochemical analysis of leaf extracts of A.alnifolia

Samples	**Expressed in terms of standards mg/ml**		
	Total phenol [1]	**Flavonoid [2]**	**Protein[3]**
Methanolic extract	18.27 ± 0.07	5.45 ± 0.03	14.28±0.14
Aqueous extract	14.03±0.37	2.22±0.25	12.84±0.16

1. Gallic acid, 2. Phloroglucinol and 3. Bovine albumin equivalent in mg/g of the dried extract.

VI. DISCUSSION

Free radicals have aroused significant interest among scientists in the past decade. Their board range of effects in biological systems has drawn the attention of many experimental works. It has been proved that these mechanisms may be important in the pathogenesis of certain diseases and ageing. There are many reports that support the use of antioxidant supplementation in reducing the level of oxidative stress and in slowing or preventing the development of complications associated with diseases. Many synthetic antioxidant components have shown toxic and/or mutagenic effects, which have shifted the attention towards the naturally occurring antioxidants. Numerous plant constituents have proven to show free radical scavenging or antioxidants activity. (Rose *et al*., 1982).

The presence of reductants in the extracts causes the reduction of Fe^{3+} / Ferric cyanide complex to ferrous form. The yellow colour of the test solution changes to various shades of green and blue depending upon the reducing power of the sample. The reducing power increased with increasing the amount of the extracts. The reducing capacity of compound serves as a indicator of its potential antioxidant activity.

The absorbance of the aqueous extracts of leaves of *A.alnifolia* were found to be higher. Increased absorbance of the extract indicates the increased reducing power. Antioxidant donates protons to the radical (DPPH), the absorption decreases. The decrease in absorption is taken as measures of extend of radical scavenging. The aqueous and methanolic extracts of leaves was subjected to screening for their possible antioxidant activity. All the extracts shows some degree of free radical scavenging activity. The aqueous and methanol extracts of leaves seems to be fairly significant.

Phytochemicals are compounds found in plants that are not required for normal functioning of the body, but have a beneficial effect on health or play an active role in amelioration of diseases. In fact, some people claim that many of the diseases afflicting human beings are the result of lack of phytonutrients in their diet. Phytonutrients have various health benefits, for example, they may have antimicrobial, anti-inflammatory, cancer preventive, antidiabetic and antihypertensive effects to mention but a few. The phytochemical constituent of a plant will often determine the physiological action on the human body (Pamplona-Roger, 1998).

In agreement with our results, the aqueous extract of the *piper bettle* leaves were found to possess maximum DPPH quenching activity (Dasgupta and De, 2004). The aqueous extract of herb *Artemisa campestris* and leaves of *Siamese neem* tree (Sithissaran *et al*., 2006) and the methanol extract of *Leea indica* and *Spermacoce articularis* (Saha *et al*., 2004) showed strong DPPH radical scavenging activity.

The phytochemical screening of the aqueous and leaf methanolic extracts of *A.hispida* revealed the presence of phenolics, flavonoids, glycosides, steroids, saponins, phlobatannins, and hydroxyanthraquinones (Iniaghe *et al*, 2010; Okorondu *et al*, 2009). The antifungal properties of extracts of leaves of *A. hispida* have also been established (Ejechi and Soucey, 1999).

Based on the results, the present study is strongly suggests that the plant *Acalypha alnifolia* leaves can be used in medicinal preparations to combat the disorders caused by oxidative stress. In future studies we can formulate a new methodology for isolation and purification of the antimicrobial compounds present in *A.alnifolia* which may act as a drug to control a broad range of human pathogens.

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