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# Antioxidant activity of *Erigeron Karvinskianus* DC. and *Ageratina Adenophora* (Spreng.) King (leaves)

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**Abstract**
Phytochemical screening of solvent and aqueous extracts clearly showed the presence of certain important secondary metabolites in *E. karvinskianus* and *A. adenophora*. Like flavonoids, alkaloids, tannins, saponins, steroids and terpenoids. The antioxidant activity of the plant extracts were determined by using phospho-molybdenum assay, Nitric oxide free radical scavenging assay and FRAP assay. The results of the present study clearly indicated that aqueous extract showed better free radical antioxidant power assay (FRAP) in *A. adenophora* at concentration of 1000 mg/ml of extract. Further, the results of the nitric oxide radical scavenging assay and phosphomolybdenum assay also showed that the aqueous and ethanol extract of *E. karvinskianus* exhibited the highest rate of antioxidant activity.

**Keywords:** Phytochemical screening, Antioxidant activity, *Erigeron karvinskianus*, *Ageratina adenophora* Phospho-molybdenum assay, Nitric oxide free radical scavenging assay and FRAP assay

## 1. Introduction
India has a rich culture of medicinal herbs and spices, which includes about more than 2000 species and has a vast geographical area with high potential abilities for Ayurveda, Unani, Siddha traditional medicines but only very few have been studied chemically and pharmacologically for their potential medicinal value. Starting from the ancient time, medicinal plants have been used to prevent and treat various health problems. Plants are still an independent source of medication in the contemporary health care delivery system. Their role is twofold in the development of medicines and served as a natural blue print for the development of new drugs.

Approximately 80% of the world population depends exclusively on plants for their health and healing. Whereas in the developed world, reliance on surgery and pharmaceutical medicine is more usual but recent years, more and more people are complementing their treatment with natural supplements [1]. Furthermore, motivation of people towards herbs are increasing due to their concern about the side effect of drugs, those are prepared from synthetic materials. The people want to concern their own health rather than merely submitting themselves to impersonal health care system. Many botanical and some common dietary supplements are good sources of antioxidants and anti-inflammatory compounds [2]. To drive the maximum health benefits, sufficient amounts of phytochemicals from a variety of plant source such as fruits, leaves and whole grain based foods are recommended.

Antioxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress. Antioxidants, both exogenous and endogenous, whether synthetic or natural, can be effective in preventing free radical formation by scavenging them or promoting their decomposition and suppressing such disorders. Due to the recent trends in nutrition towards development of healthy foods in the form of 'functional foods', one of the desirable properties in a dietary component is considered to be its antioxidant effect [3].

Currently available synthetic antioxidants like Butylated Hydroxy Anisole (BHA). Butylated Hydroxy Toluene (BHT), Tertiary Butylated Hydroquinon. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Moreover, these synthetic antioxidants also show low solubility and moderate antioxidant activity. In recent times natural antioxidants have attracted considerable interest among nutritionists, food manufactures and consumers, food manufactures and consumers, due to their presumed safety and potential therapeutic value.

Antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. The Food and Nutrition Board of the National Academy of Science defined a dietary antioxidant as a substance in foods that significantly decreases the adverse effects of reactive oxygen species, reactive nitrogen species, or both on normal physiological function in humans. In order to prolong the storage of foods, several synthetic antioxidants such as Butylated Hydroxy Toluene (BHT) and Butylated Hydroxy Anisole (BHA) are used currently, but these substances may be inappropriate for chronic human consumption, as recent publications have mentioned their possible toxic properties for human health and the environment [4].

All parts of *E. karvinskianus* (Asteraceae) has medicinal value. Which is utilized in traditional system of medicine. I'ts leaves are taken externally, to cure skin diseases. Traditionally used as indigestion, enteritis, epidemic hepatitis, and hematuria. Previous investigation revealed that this plant contains monoterpenoids, sesquiterpenoids, diterpenoids, triterpenoids, and phenolic derivative.

*Ageratina adenophora*, commonly known as 'sticky snakeroot', belonging to the family Asteraceae. *A. adenophora* leaf used to cure itching, skin disease, wounds, measles. It act as antibacterial, antifungal and antioxitant activity. It contains various group of phytoconstituents viz. Alkaloids, flavonoids, steroids and trepenoids.

With the view of the aforesaid background, in the present study

was carried out with the following objectives.

1. To extract the phytochemical components from the leaves of *Erigeron karvinskianus* and *Ageratina adenophora* using aqueous and solvents extracts.
2. To find out the different phytochemical constituents in the extract of selected medicinal plants through phytochemical screening.
3. To evaluate the antoxidant activity of crude extracts using Phospho – Molybdenum assay, Nitric oxide free radical scavenging assay, and FRAP assay.

## 2. Materials and methods

### 2. 1 Chemicals

Sulfuric acid, Sodium phosphate, Ammonium molybdate, Ascorbic acid Sodium Nitropurusside, Phosphate buffer, Griess reagent (2% ortho phosphoric acid, 1% sulphnilamide, 0.1% N-1 napthyl ethylene diamine), TPTZ, HCL, $Fecl_3$ and $6H_2O$ were used and the absorbance values were measured as for sample solutions.

### 2.2 Plant materials

Fresh leaves of *Erigeron karvinskianus* DC. and *Ageratina adenophora* (spreng.) King. were collected from the natural strands of Avalanchi forest in Nilgiri district, Tamil Nadu. Fresh plant materials were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

### 2.3 Preparation of Extracts

#### 2.3.1 Solvent extraction

Fifty grams of air-dried powder was taken 200 ml of petroleum ether/ Ethyl acetate/ Ethanol a conical flask, plugged with cotton wool and they were shaken at room temperature for 2 days. After 2 days the supernatant was collected and the solvent was evaporated to make the final volume into one fourth of the original volume and stored at 4˚ C in airtight bottles.

#### 2.3.2 Aqueous extraction

Fifty grams of air-dried powder was taken in a conical flask containing 200ml of water and the flask is plugged with cotton wool. Then, they were shaken at room temperature for 2 days. After 2 days the supernatant was collected and the solvent was evaporated to make the final volume into one fourth of the original volume and stored at 4˚ C in airtight bottles.

The percentage yield of the extracts from each solvent extraction was calculated. The crude extract was crushed into powder and then kept in desiccators.

### 2.4 Phytochemical screening

Phytochemical screening of solvent and aqueous extract of *E. karvinskianus* and *A. adenophora* were carried out by using standard procedure to identify the constituents by Trease and Evans [5] methods.(Alkaloids, Flavonoids, Tannins, Saponins, Steroids)

### 2.5 Antioxidant activity

The antioxidant activity of crude extracts was determined by the following *in-vitro* methods.

#### 2.5.1 Phospo-Molybdenum assay

The total antioxidant capacity of the extracts were evaluated by the phospo-molybdenum Mo (V) by the extract and subsequent formation of green phosphate /Mo complex at acidic pH.0.3ml each extract (6%) in triplicates were combined with 3 ml of reagent solution (0.6) method described by [6]. The assay is based on the reduction of Mo (VI) to sulfuric acid, 28mM sodium phosphate and 4mM Ammonium molybdate). The absorbance of the reaction mixture was measured at 695 nm using a spectrophotometer against blank after cooling to room temperature. Methonal (0.3ml) in the place of extract was used as the blank. The antioxidant activity is expressed as the number of gram equivalent of ascorbic acid. The calibration curve was prepared by mixing ascorbic (1000, 500, 250, 125, 62.5 and 31.25µg/ml) with methanol.

#### 2.5.2 Nitric oxide free radical scavenging Assay

Nitric oxide free radical scavenging was carried out as follows. Sodium nitropurusside (10Mm) in phosphate buffer (pH 7.7) was incubated with 900µg/ ml of each extract (6%) dissolved in their respective solvents in triplicate, and the tubes were incubated at 25 for 120 min. After incubation, 0.5 ml of the rection mixture was diluted with 0.5 ml of griess reagent (2% ortho phosphoric acid, 1% sulphnilamide, 0.1% N-1 napthyl ethylene diamine). Ascorbic acid was used as the standard. The absorbance of the pink chromophore formed during diazotization of nitrite with sulphanilamide and subsequent coupling with N-1 napthyl ethylene diamine was measured at 346 nm against the corresponding blank solution. Difference in absorbance between the control and sample and expressed as percentage free radical scavenging of the nitric oxide by the extract [7].

$$\text{Radical scavenging activity (\%)} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

#### 2.5.3 Ferric Reducing Antioxidant Power assay (FRAP)

3.6 mL of FRAP solution (0.3 M of Acetate buffer – pH 3.6; 10 Mm of TPTZ in 40 mM of HCL and 10 mM of $Fecl_3$ $6H_2$ O) is added to distilled water (0.4 ml) and incubated at 37°C for 5 min. Then this solution mixed with certain concentration of the plant extract (80 ml) which was measured at 593 nm. For construction of the calibration curve, five concentrations of $feSO_4$, 6H2O (0.1, 0.4, 0.8, 1, 1.12, 1.5 m M) were used and the absorbance values were measured as for sample solutions [8].

#### Statistical analysis

Results of the research were analyzed for statistical significance by ANOVA. This research was performed by three duplicates with replicate.

## 3. Results

### 3.1 Phytochemical screening

Ethnol and aqueous extracts of the selected samples were subjected to qualitative organic analysis (Table 1)

**Table 1:** Preliminary phytochemical screening of *E. karvinskianus* and *A. adenophora*[*]

| S. No | Phytoconstituents | *E. karvinskianus* | | | | *A. adenophora* | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | PE | E A | Ethanol | Aqueous | PE | EA | Ethanol | Aqueous |
| 1 | Alkaloids | -- | -- | -- | -- | -- | -- | -- | + |
| 2 | Tannins | -- | -- | -- | -- | -- | -- | + | -- |
| 3 | Saponins | + | + | + | + | + | -- | + | + |
| 4 | Flavonoids | + | -- | -- | -- | + | -- | -- | -- |
| 5 | Steroids | + | -- | -- | -- | + | + | + | -- |
| 6 | Terpenoids | -- | -- | -- | -- | + | -- | -- | + |

[*]**(+)** Indicates Positive **(--)** Indicates Negative

### 3.2 Antioxidant activity

#### 3.2.1 Phospho-molybdenum assay

The result of total antioxidant activity (TAC) by phosphor-moltbdenum assay is shown in the Table 2. It is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate /Mo (V) complex at acidic pH. TAC of the phospho- molybdenum model evaluates both water-soluble and fat-soluble antioxidant capacity (total antioxidant capacity). The results indicate a concentration dependent total antioxidant capacity. The extracts of all the tested plant specimens possess total antioxidant activity, but varying degrees, ranging from 3.987 to 7.675 mg/ml. using the organic solvent extraction, generally ethanol showed better TAC. A maximum total antioxidant activity was offered by ethanol extract of *E. karvinskianus* (7.675 mg/ml), followed by ethanol extract of *A. adenophora* (7.319 mg/ ml), Aqueous extract of *A. adenophora* (4.007 mg/ ml) and aqueous extract of *E. karvinskianus* (3.987 mg/ ml) at 1000 mg/ ml concentration (Table 2).

**Table 2:** Phospho–Molybedenum assay in ethanol and aqueous extract of *E. karvinskianus and A. adenophora**

| Plant Species | Total antioxidant activity (mg Ascorbic acid equivalent / ml sample) | |
|---|---|---|
| | Plant extracts | |
| | Ethanol | Aqueous |
| *Erigeron karvinskianus* | 7.67 ± 0.038 | 3.98 ± 0.377 |
| *Ageratina adenophora* | 7.31 ± 0.112 | 4.07 ± 0.051 |

*Data are mean of three replicates, ± standard error.

#### 3.2.2 Nitric oxide free radical scavenging assay

The nitric oxide free radical scavenging assay of plant sample was given in Table 3, radical scavenging activity was found to be ranged from 40.48 to 57.34%. Among the two different plants, the aqueous extract of *E. karvinskianus* (57.34%) have exhibited the highest rate of free radical scavenging activity followed by 55.06 of *A. adenophora* in aqueous extract.

**Table 3:** Nitric oxide free radical scavenging assay in ethanol and aqueous extracts of *E. karvinskianus and A. adenophora**

| Plant Species | Radical scavenging activity (%) | |
|---|---|---|
| | Plant extracts | |
| | Ethanol | Aqueous |
| *Erigeron karvinskianus* | 50.45 ± 0.750 | 57.34 ± 2.459 |
| *Ageratina adenophora* | 40.48 ± 2.706 | 55.06 ± 1.214 |

*Data are mean of three replicates, ± Standard error

#### 3.2.3 FRAP assay

The total antioxidant activity of the plant sample is shown in the table 4. The extracts showed considerable antioxidant activity ranging from 326.487 to 339.976. The maximum total antioxidant activity was observed in aqueous extract of *A. adenophora*.

**Table 4:** FRAP assay in ethanol and aqueous extract of *E. karvinskianus and A. adenophora*

| Plant Species | Total antioxidant activity (mg $FeSO_4$ equivalent / g sample) | |
|---|---|---|
| | Plant extracts | |
| | Ethanol | Aqueous |
| *Erigeron karvinskianus* | 337.43 ± 0.731 | 338.68 ± 0.978 |
| *Ageratina adenophora* | 326.48± 0.838 | 339.97± 0.050 |

*Data are mean of three replicates, ± standard error.

### 4. Discussion

Plant materials containing phenolic constituents are increasingly of interest for probing as they retard oxidative degradation of lipids and there by improve the quality and nutritional value of food. The importance of the antioxidant constituents of plant material in the maintenance of health and protection from

coronary heart disease and cancer has been will recognized [2]. It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process [9] compounds are a class of antioxidant agents which act as free radical terminators [10].

Free radicals contribute to more than one hundred disorders in human including atherosclerosis, arthritis, ischemia and repercussion injury of many tissues. Central nervous system injury, gastritis, cancer and AIDS [11]. Antioxidants through their scavenging power are useful for the management of those diseases. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts. Antioxidants, both exogenous, whether synthetic or natural, can be effective in prevention of the free radical formation by scavenging or by effecting promotion of their decomposition and suppression of such disorders [12].

### 4.1 Phytochemical screening

All plant parts synthesize some chemicals by themselves, to perform their physiological activities. In our present study, the investigated plants have exhibited different kinds of secondary metabolites. The medicinal value of these secondary metabolites is due to the presence of chemical substances that produce a definite physiological action on the human body. The most important of these substances include, alkaloids, glucosides, steroids, flavonoids, fatty oils, resins, mucilages, tannins, gums, phosphorus and calcium for cell growth, replacement, and body building [13].

Alkaloids have been well investigated for many pharmacological properties including antiprotozoal, cytotoxic and antidiabetic [14] and anti-inflammatory properties, but there are only few reports about their antimicrobial properties. Plants with alkaloids such as *Meliah azadiarch***,** *Abutilon indicum* and *Calotropis gigantean* are used to treat asthma.

Saponins are glycosides occurring widely in plants. They are abundant in many foods consumed by animals and man. Saponin is used as mild detergents and in intracellular histochemistry staining to allow antibody accessto intracellular proteins. In medicine, it is used in hypercholesterolemia, hyperglycemia, antioxidant, anti-cancer, anti-inflammatory [15], central nervious system activities [16]. It is also known to have antifungal properties. The plants having saponins are *Meliah azadiarch* and *Calotropis gigantean.*

Plant steroids are known to be important for their cardiotonic activities, possession of insecticidal, anti-inflammatory [17], analgesic properties [18], central nervous system activites and antimicrobial properties. They are also used in nutrition, herbal medicine and cosmetics. Out of the six plants, studied steroids are present in *Meliah azadiarch* and *Calotropis gigantea* [16].

Tannins were reported to exhibit antidiabetic [14], anti-inflammatory [19], antibacterial and antitumor activities. It has also been reported that certain tannins were able to inhibit HIV replication selectively besides use as diuretics. Plant tannins have been widely recognized for their pharmacological properties and are known to make trees and shrubs a different meal for many caterpillars [20].

### 4.2 Antioxidant activity

#### 4.2.1 Phospho-Molybdenum assay

Phospho-Molybdenum assay based on the reduction of MO (VI)-MO (V) by the extract and formation of a MO (V) complex at acidic PH. Increase in absorbance was observed by standard and extract. The total antioxidant activity of crude extracts of two plant specimens were ranged between 3.987 to 7.675 mg/ml. These value are found to be higher than that of the earlier report in *Caesalpinia bonducella* [21].

#### 4.2.2 Nitric oxide free radical scavenging assay

Natural antioxidants that are present in herbs are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. Herbs contain free radical scavengers like polyphenols, flavonoids and phenolic compounds. In the present study, we have evaluated the free radical scavenging activity of ethanol and aqueous extracts of *E. karvinskianus* and *A. adenophora* leaves. Among the four extracts and standard tested for the *in vitro* antioxidant activity using the Nitric oxide free radical scavenging method, the crude ethanolic extracts of *A.adenophora* and *E.karvinskianus* leaves showed antioxidant activity of 40.48 ± 2.70% and 50.45 ± 0.75 % respectively. Similarly the aqueous extracts of *A. adenophora* and *E. karvinskianus* leaves showed antioxidant activity of about 55.006 ± 1.21% and 57.34 ± 2.45% respectively. These value are found to be higher than that of the earlier report in *Ficus deltoidea* [22].

#### 4.2.3 FRAP assay

Most of the extracts showed a considerable antioxidant effect from 326.487 ± 0.83 mg of $FeSO_4$ / g dry plant sample equivalents in *A. adenophora* leaves (Ethanol extract) to339.976 ± 0.05 mg of $FeSO_4$ /g dried sample in *A. adenophora* (Aqueous extract). As it was shown (Table- 4), all the plant samples possessed antioxidant properties, where the aqueous extracts of *E. karvinskianus* leaves showed total antioxidant activity of 338.687 ± 0.978mg $FeSO_4$ /g. These value are found to be higher than that of the earlier report in *Caesalpinia bonducella* [21].

### 5. Conclution

In nature all the plants synthesis phytochemicals to perform defense activities. In present study, the investigated plants have exhibited different kinds of secondary metabolites. Solvent and aqueous extracts of leaf samples were determined phytochemical constituents and antioxidant activity. Hence, the reults of the study, clearly explained that among the two plant material, the *A. adenophora* was found to be more potential than the *E. karvinskianus*. Thus, after conducting certain advanced studies, the phytochemical compounds may be saparated and biosythesis and structural elucidations can be carried out in future for the effective utilization of these potential medicinal plants.

### 6. References


1. Dursum ES, Akcicek E. Herbes as Food source in Turkey. *Asia pacific J. Cancer prev*. 2004; 5:722-733.
2. Khalail MY, Moustafa AA, Naguib NY. Growth, phenolic compounds and antioxidant activity of some medicinal plants grown under organic farming condition. World J. Agri. Sci. 2007; 3(4):451-457.
3. Akanksha Mirshra, Bapat Mirnal M, Tilak Jai Devasagayam C, Thomas PA. Antioxidant activity of *Garciniaindica* and its syrup. *Cureent sci*. 2006; 91(1):0-93.
4. Nan Wu, Kuang Fu, Yu-Jie Fu, Yuan-Gang Zu, Fang-Rong Chang, Yung-Husan Chen, *et al*, Antioxidant activities of

extracts and Main components of Pigeonpea *Cajanus cajan* L. Millsp. Leaves. Molecules. 2009; 14:1032-1043.
5. Trease GE, Evans WC. Pharmacognosy 11th Edn. Brailliar Tridel and Macmillian Publishers, London, 1989.
6. Prieto P, Pineda M, Anguilar M. Spectrophotometeric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex. Specific application to the determination of Vitamin E. *Anal. Biochem* 1999; 269:337-341.
7. Hou D, Larsen K, Larsen SS. Caesalpiniaceae. *Flora Malesiana*, Series 1, 1996; 12(2):409-730.
8. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power, The FRAP assay. *Anal. Biochem.* 1996; 239:70-76.
9. Kessler RC, Barker PR, Colpe LJ, Epstein JF, Gfroerer JC, Hiripi E *et al*. Screening for serious mental illness in the general population archives of general psychiatry. 2003; 60(2):68-74.
10. Shahidi F, Wanasunndara PKJPD. Phenolic antioxidants. Crit. Rev. Food Sci. Nut. 1992; 32:67-103.
11. Pourmorad F, Hosseinimehr SJ, Shahabimajd N. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *Afr. J.Biotech.* 2006; 5(11):1142- 1145.
12. Souri E, Amin G, Farsam H, Barazandeh Tehrani M. Screening of antioxidant activity and phenolic contant of 24 medicinal plant extracts. *DARU.* 2008; 16:283-287.
13. Kubmarawa D, Khan ME, Punah Hassan AM. Phytochemical screening and antibacterial activity of extracts from *Parkiaclapper tonianakeay against* human pathogenic bacteria. J. Med. Pl. Res. 2008; 2(12):352-355.
14. Cherian S, Augusti KT. Insulin sparing action of leucopelargonidin derivative isolated from Ficusben ghalesis Linn. Indian J. Exp. Biol. 1995; 33:608-611.
15. Manach C, regerat F, Texier O. Bioavaillability, metabolism and physiological impact of4-oxo-flavonoids.*Nutr. Res.* 1996; 16:517-544.
16. Argal A, Pathak AK. CN Sactivity of *Calotropisgigantearoots*. *J. Ethnopharmacol*. 2006; 106:142-145.
17. Akindele AJ, Adenyemi OO. Anti-inflammatory activity of the aqueous leaf extracts of *Byrsocarpuscoccineus. Fitoterapia*, 2007; 78:25-28.
18. Malairajan P, Geetha G, narasinhan S, Jessi Kala Veni K. Analgesic activity of some Indian medicinal plants. J. *Ethnopharmacol*. 2006; 19:425-428.
19. Latha RH, Geetha T, Varalakshmi P, Effect of *Vernonia cinera* less flower extract in adjuvant induced arthritis. *Gen. Pharmacol*. 1989; 31:601-606.
20. Haslem E. Plant polyphenols: vegetable tannins revisited-chemistry and pharmacology of natural products. Cambridge University Press. 1989, 169 (Original not seen).
21. Rajalakshmi P, Vadivel V, Subhashini G, Pugalenthi M. Ptytochemical Screening and *in vitro* Antioxidant activity of Caesalpinia bonducella. Indian Journal of Appiled Research. 2015; 5:(11).
22. Misbah H, Aziz AA, Aminudin N. Antidiabetic and Antioxidant properties of ficus deltoidea fruit extracts and fractions. BMC Complement Altern Med. 2013; 13:118.