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# AN ASSESSMENT OF IN –VITRO ANTI-OXIDANT POTENTIAL OF SELECTED MEDICINAL PLANTS EXTRACTS

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## ABSTRACT

The aim of the study was to evaluate the antioxidant activity of leaf extracts of five plant species selected on the basis of their reported ethno botanical uses. The alkaloid and flavonoid fraction of plant extracts was analyses against L-ascorobic acid by DDPH assay. The potential of L- ascorobic acid to scavenge DPPH (1,1-diphenyl-2-picrylhydrazyl) radical become almost stable after 70 or 80%. The fractions showed dose dependent free radical scavenging property. The alkaloids and flavonoids fraction of *Abutilon indicum*, leaf extract were found to 75.99% (ethanol), 79.16% (methanol), 75.04% (aqueous) and 77.02 %, 81.07%, 47.54%, respectively. The *Adathoda visca* leaf extracts were found to be 75.17%, 81.07%,71.32% and 79.16%, 85.10%, 44.32%, *Datura stromonium* 73.21%, 82.86%, 70.27% and 70.43%,75.46%, 41.17% respective *Lantana camara* fraction showed 70.22%, 80.92%, 70.50% and 68.47% 80.90%,37.72% activities and *Tridax procumbens* 72.07%,79.34%,68.80% and 62.27% 62.67%, 33.02%. On comparative basis flavonoids leaf extracts of *Adathoda visca* and *Datura stromonium* fractions exhibit amazing antioxidant properties that support their traditional use as natural antioxidant.

**KEYWORDS:** Antioxidant activity, Ascorbic acid, DPPH assay, Medicinal plants.

## INTRODUCTION

Cancer is a complex group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body with many possible causes (**Rathore *et al.*, 2010**), including genetic factors, lifestyle factors such as tobacco use, diet, and physical activity, certain types of infections and environmental exposures to different types of chemicals and radiation (**Balachandran *et al*,. 2005**).

Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen. Examples include peroxides, superoxide, hydroxyl radical, and singlet oxygen. The potentially reactive derivatives of oxygen, attributed as reactive oxygen species (ROS), are continuously generated inside the human body.

The generated ROS are detoxified by the antioxidants present in the body. However, overproduction of ROS and or inadequate antioxidant defense can easily affect and persuade oxidative damage to various biomolecules including proteins, lipids, lipoproteins and DNA. This oxidative damage is a critical etiological factor implicated in several chronic human diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis and neurodegenerative diseases and also in the ageing process.

The antioxidants are referred as a substance, which have the capacity to neutralize free radicals by donating one of their own electrons. Antioxidants prevent oxidative damage caused by free radical, it can inhibit with the oxidation process by reacting with free radicals. Antioxidants act in different ways by preventing free radical formation by chelating, catalytic metals and also by actingas oxygen scavengers (**Braca *et al*, 2001**).

Natural antioxidant (food antioxidant) are structurally phenolic or polyphenolic compounds commonly occurring in plant materials, that interfere with the formation of free radicals, hence prevents the formation of peroxide.

The antioxidants occurring naturally are present in all foods and herbal medicines. Their addition in small quantities, within permissible limits, delay, retard or prevent development of rancidity caused by the atmospheric oxygen and thus preserve fats and oils, carotenoids and other nutritive ingredients of food.

Synthetic antioxidants are phenolic compounds that perform the function of capturing free radicals and stopping the chain reaction. Compounds include: hydroxylated anisole (BHA) Butylated hydroxylated toluene (BHT). Synthetic antioxidants are cheap though natural antioxidants are also attractive as additives in food items .Superiority of Natural Antioxidants over Synthetic ones have been proven in terms of safety, tolerance, and non-toxicity (**Emad Mohamed *et al*,.2014**).

The natural antioxidants have acquired much importance in human health in recent times. Plants are potential sources of natural antioxidants that have immense therapeutic value. Many complex diseases like atherosclerosis, stroke, diabetes and cancer can be treated and prevented with antioxidant-drugs (**Devasagayam *et al.*, 2004**). The medicinal plants that show significant antioxidant activity include*Abutilon indicum,Adhatoda viscaDatura stromonium*, *Lantana camara*and *Tridax procumbens*were screened for antioxidant activities using *in-vitro* scavenging of free radicals by DPPH against ascorbic acid as standard antioxidant. Many other plant species have been investigated in the search for novel antioxidants (**Buyukokuroglu 2001**) but generally there is still a demand to find more information regarding the antioxidant potential of plant species as they are safe and also bioactive. Therefore, in recent years, considerable attention has been focused towards the identification of plants with antioxidant ability. Flavonoids and alkaloids are widely distributed secondary metabolites with antioxidant and antiradical properties. (**Aqil *et al*,. 2006**). Thispaper reports the*in-vitro* antioxidant activity of selected medicinal plant extracts for potential application as anticancer agent.

## 2. MATERIALS AND METHODS

### 2.1 Chemicals

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) was obtained from Sigma Aldrich Co., St. Louis, USA. All other chemicals used were of analytical grade.

### 2.2 Collection of plant material

On the basis of ethnobotanical available literature and visual observation of plants that were relatively free from diseases and insect damages five plant species, *Adathoda visca*, *Datura stramonium*, *Lantana camara*, *Tridax procumbens*, and *Abutilon indicum* have been selected for the present study .The collected plant material were thoroughly washed and then dried under shade at 25± 2°C for about10 days. The dried plant samples were ground well into a fine powder in a mixer grinder. The powdered samples were then stored in air tight containers at room temperature.

### 2.3. DPPH scavenging activity

The free-radical scavenging capacity of different extracts of selected plants was evaluated with the DPPH stable radical, by the methodology described by Blois (1958). Briefly, 0.1mM alcoholic solution of DPPH was prepared and 2ml of this solution was added to 0.3ml of different extract concentrations (1-100 mg/ml) and allowed to react at room temperature. After 30min, the absorbance values were measured at 517nm against a suitable blank. DPPH radical scavenging by L-ascorbic acid (0.1M) was used as a standard. The radical scavenging activity (percent inhibition) was expressed as percentage of DPPH radical elimination calculated according to the following equation:

The percentage inhibition of $DPPH^{\cdot}$ in the reaction medium was calculated by comparing with the control.

$$\% \text{ inhibitions} = \frac{A_{standard} - A_{sample}}{A_{control}} \times 100$$

## RESULTS AND DISSCUSION

DPPH assay has been extensively used for rapid screening antioxidant activity and is sensitive enough to detect active ingredients at low concentration. When DPPH radicals encounter a free radical bearing substance, it would be scavenged and the absorbance decreases. Hence, DPPH radicals are widely used to investigate the scavenging activity of some natural compounds.

In the present study, the methanol and ethanol extracts and aqueous extracts of *A.indicum, A. visca, D.stromonium, L. camera* and *T. procumbens* are investigated for their antioxidant properties. The alkaloids fraction of *A. indicum*, extracts was analyzed against L-ascorobic acid by DPPH assay. The potential of L-ascorobic acid to scavenge DPPH radical become almost stable after 70 or 80 µg/ml [Fig.1(a)]. Flavonoids extract of *A. indicum* show DPPH reduction (methanol 81.07%, ethanol 77.02% and aqueous 47.54%) at 100 µg/ml. The maximum anti-oxidant effect was found in the methanol extract i.e 81.07% µg/ml of *A. indicum* [Fig.1(b)].

Alkaloids extract of *A. visca* show DPPH reduction (methanol 81.07%, ethyl alcohol 75.17% and acetone 71.32%) at 100µg/ml. The maximum antioxidant effect was found in the methanol extract i.e 81.07% of *A. visca* [Fig.2 (a)]. Flavonoids extract of *A. visca* show DPPH reduction (methanol 85.10%, ethanol 79.16% and aqueous 44.32%) at 100 µg/ml. The maximum antioxidant effect was found in the methanol extract i.e 85.10% µg/ml of *A. visca* [Fig.2 (b)].

Alkaloids extract of *D. stromonium* show DPPH reduction (methanol 82.86%, ethanol 73.21% and aqueous 70.27%) at 100µg/ml. The maximum antioxidant effect was found in the methanol extract i.e 82.86% µg/ml of *D. stromonium* [Fig.3(a)].Flavonoids extract of *D.stronomonium* show DPPH reduction (methanol 75.46%, ethanol 70.43% and aqueous 41.17%) at 100 µg/ml. The maximum antioxidant effect

was found in the methanol extract i.e 75.46% µg/ml of *D.stronomonium* [Fig.3(b)].

Alkaloids extract of *L. camera* show DPPH reduction (methanol 80.90%, ethanol 70.22% and aqueous 70.50%) at100 µg/ml. The maximum antioxidant effect was found in the methanol extract i.e 80.90% µg/ml of *L. camera* [Fig.4(a)]. Flavonoids extract of *L. camera* show DPPH reduction (methanol 80.90%, ethanol 68.47% and aqueous 37.72%) at 100 µg/ml. The maximum anti-oxidant effect was found in the methanol extract i.e 80.90% µg/ml of *L. camera* [Fig.4 (b)].

Alkaloids extract of *T. procumbens* show DPPH reduction (methanol 79.34%, ethanol 72.07% and aqueous 68.80%) at 100µg/ml. The maximum antioxidant effect was found in the methanol extract i.e 79.34% µg/ml of *T. procumbens* (Fig. 5(a). Flavonoids extract of *T. procumbens* show DPPH reduction (methanol 62.67%, ethanol 62.27% and aqueous 33.02%) at 100 µg/ml. The maximum antioxidant effect was found in the methanol extract i.e 62.67% µg/ml of *T. procumbens* [Fig.5(b)].

**Plants: Abutilon indicum**
**Alkaloid extract**

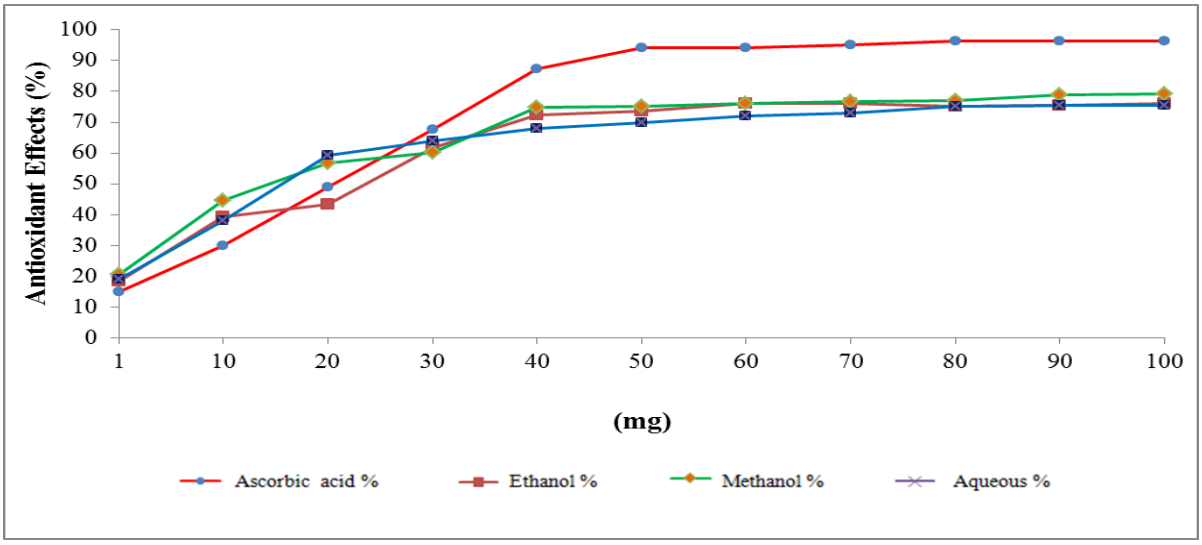


**Fig. 1(a): Free radical scavenging activity of Alkaloids extracts of *Abutilon indicum*, and L-ascorbic acid (control) was spectrophotometrically measured at 517 nm using the DPPH assay.**

**Flavonoid extract**

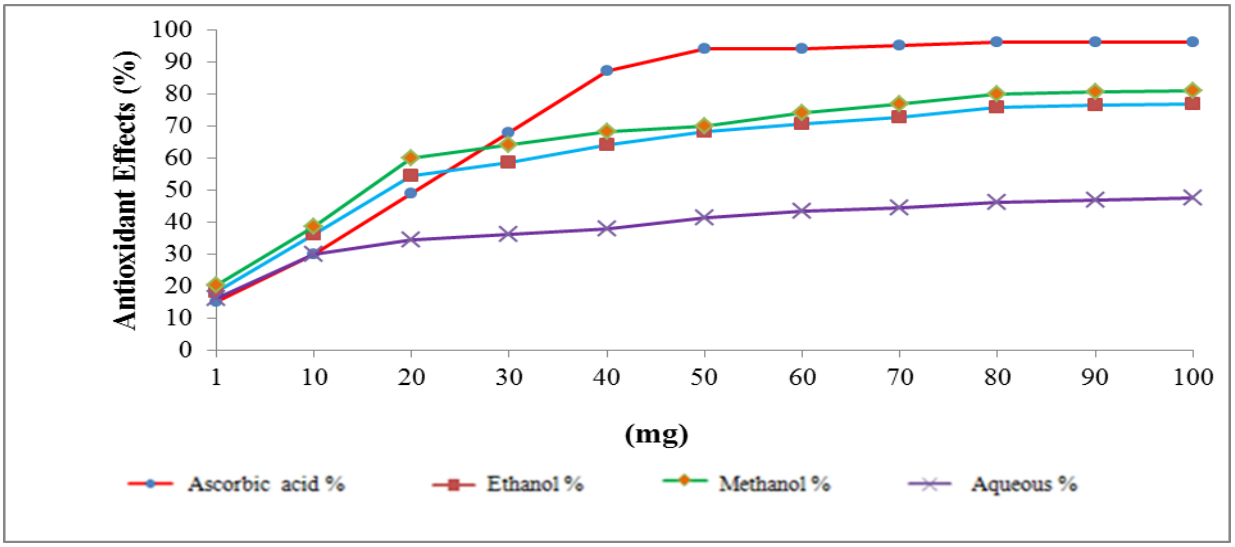


**Fig. 1(b): Free radical scavenging activity of Flavonoids extracts of *Abutilon indicum* and L-ascorbic acid (control) was spectrophoto metrically measured at 517 nm using the DPPH Assay.**

**Plants:** ***Adathoda visca***
**Alkaloid extract**

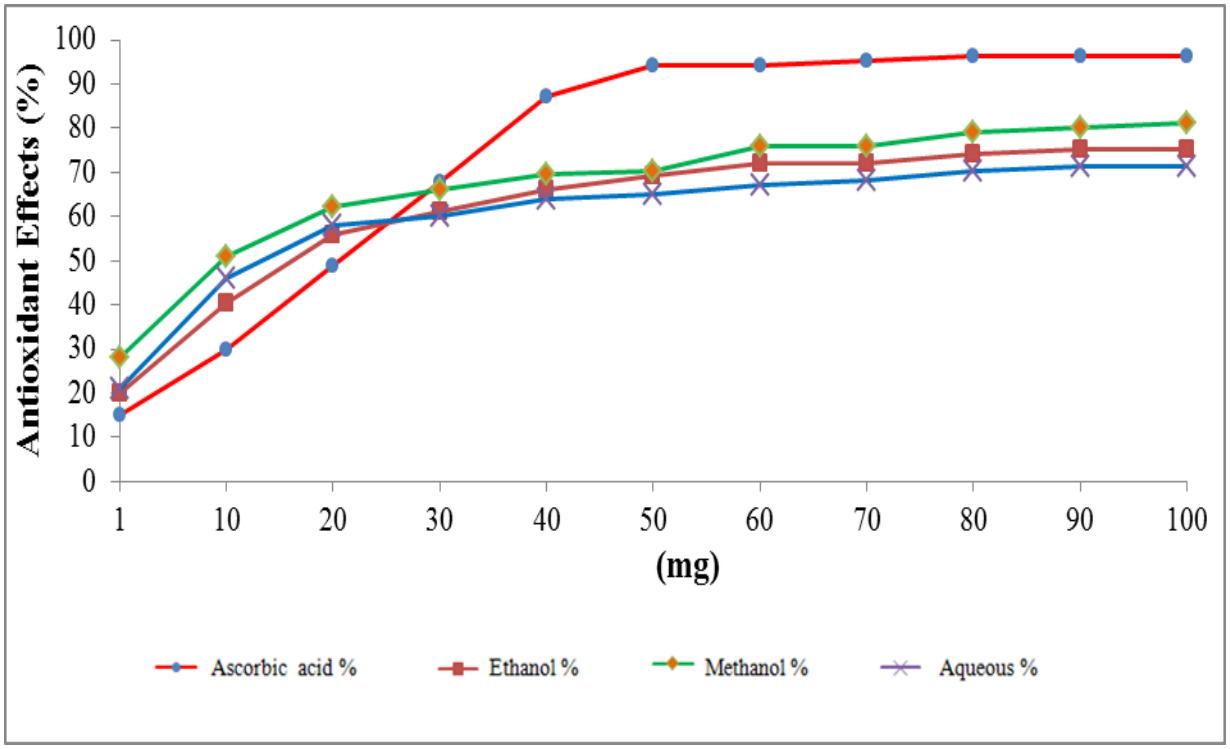


**Fig. 2 (a): Free radical scavenging activity of Alkaloids extracts of Adathoda visca and L-ascorbic acid (control) was spectrophoto metrically measured at 517 nm using the DPPH Assay.**

**Flavonoid extract**

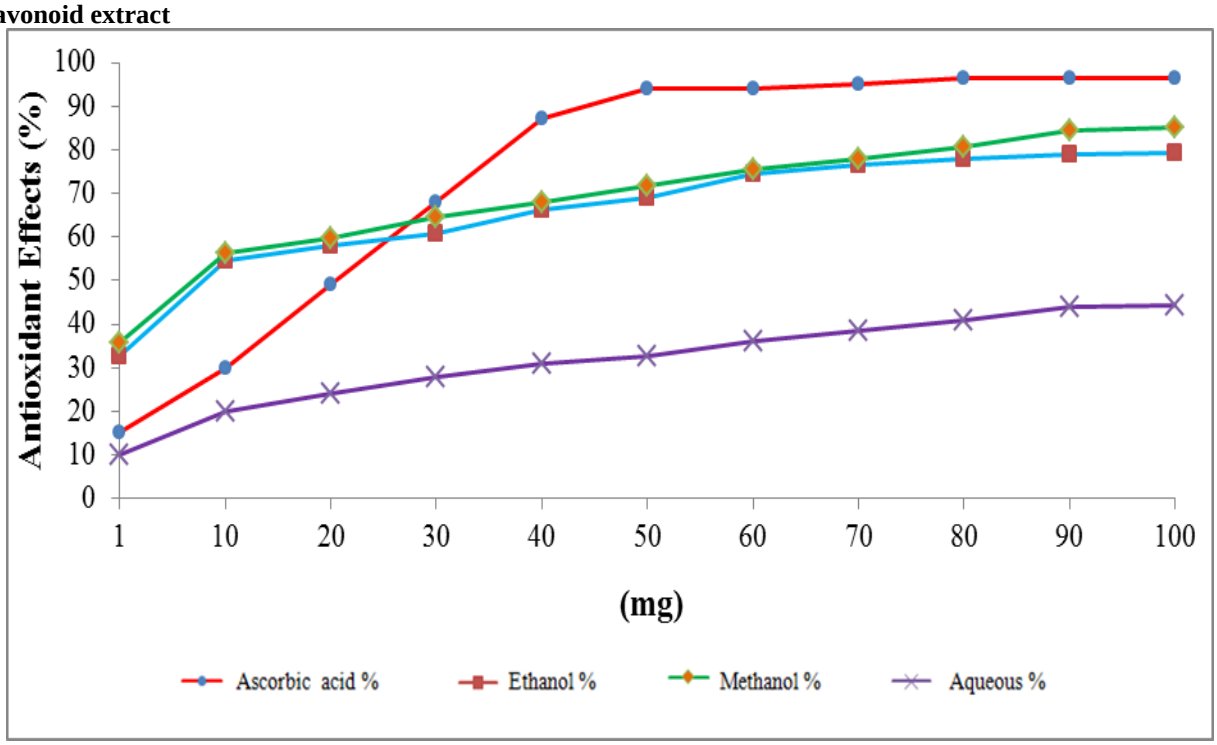


**Fig. 2(b): Free radical scavenging activity of Flavonoids extracts of *Adathoda visca* and L-ascorbic acid (control) was spectrophoto metrically measured at 517 nm using the DPPH Assay.**

**Plants: *Datura stronomonium***
**Alkaloid extract**

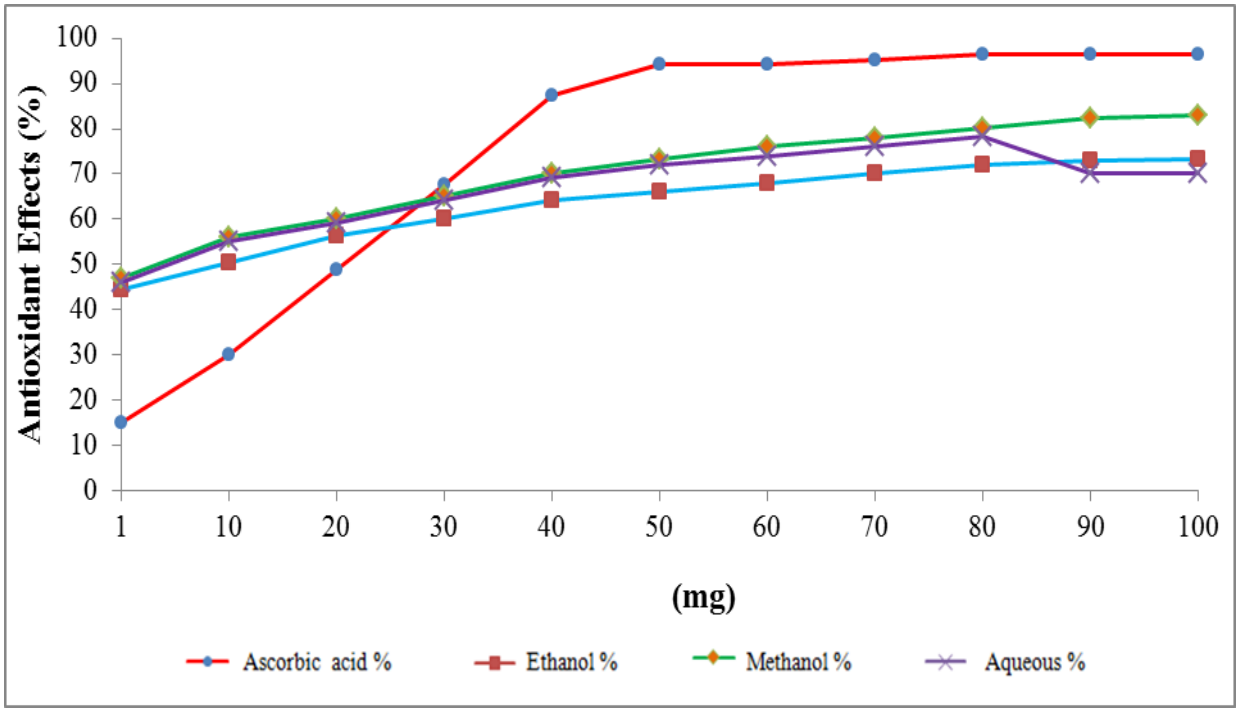


**Fig. 3(a): Free radical scavenging activity of Alkaloids extracts of *Datuara stromonium* and L-ascorbic acid (control) was spectrophoto metrically measured at 517 nm using the DPPH assay.**

**Flavonoid extract**

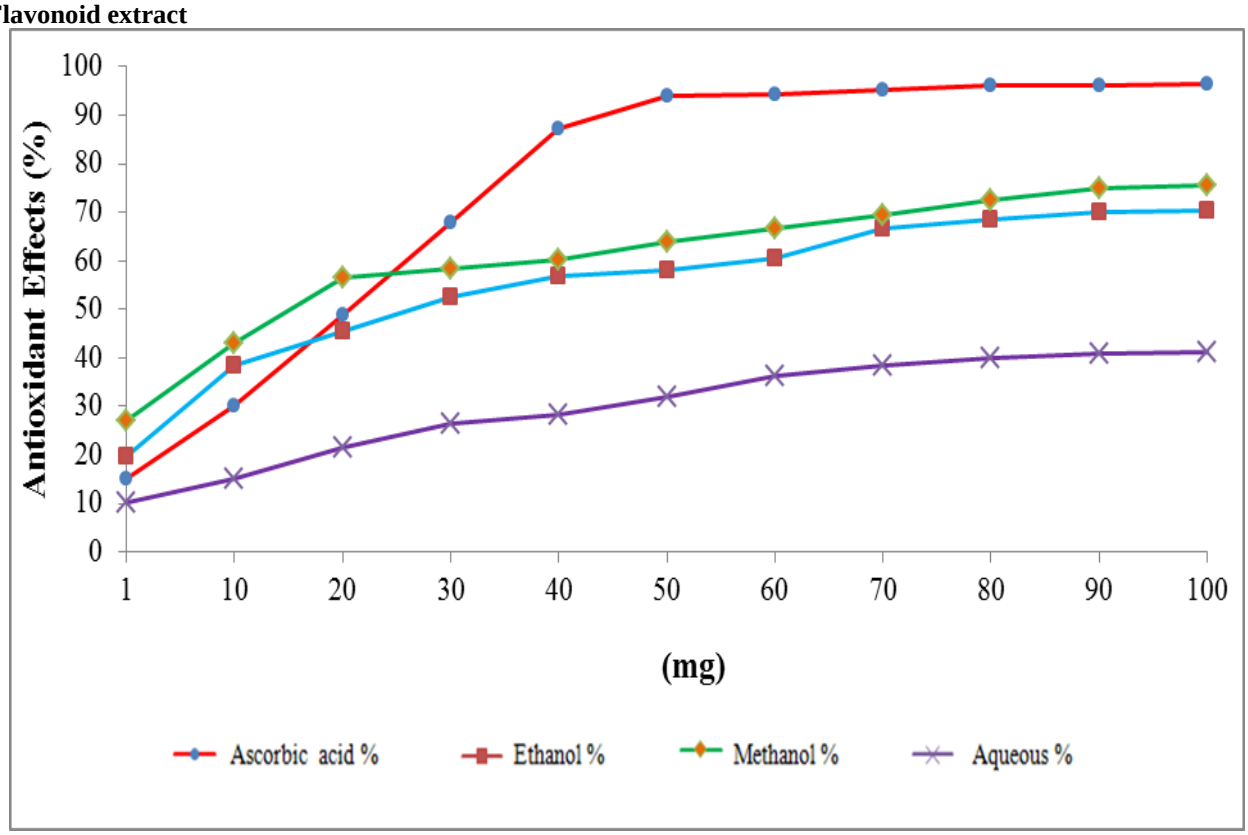


**Fig. 3 (b): Free radical scavenging activity of Flavonoids extracts of *Datuara stromonium* and L-ascorbic acid (control) was spectrophotometrically measured at 517 nm using the DPPH assay.**

**Plants:** Lantana camera
**Alkaloid extract**

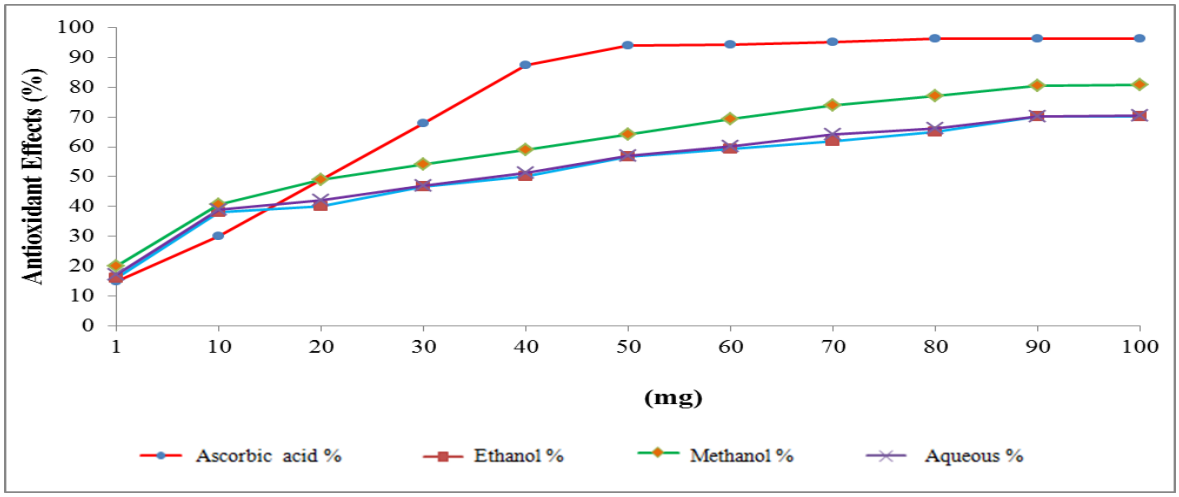


**Fig. 4(a): Free radical scavenging activity of Alkaloids extracts of Lantana cameraand L-ascorbic acid (control) was spectrophoto metrically measured at 517 nm using the DPPH Assay.**

**Flavonoid extract**

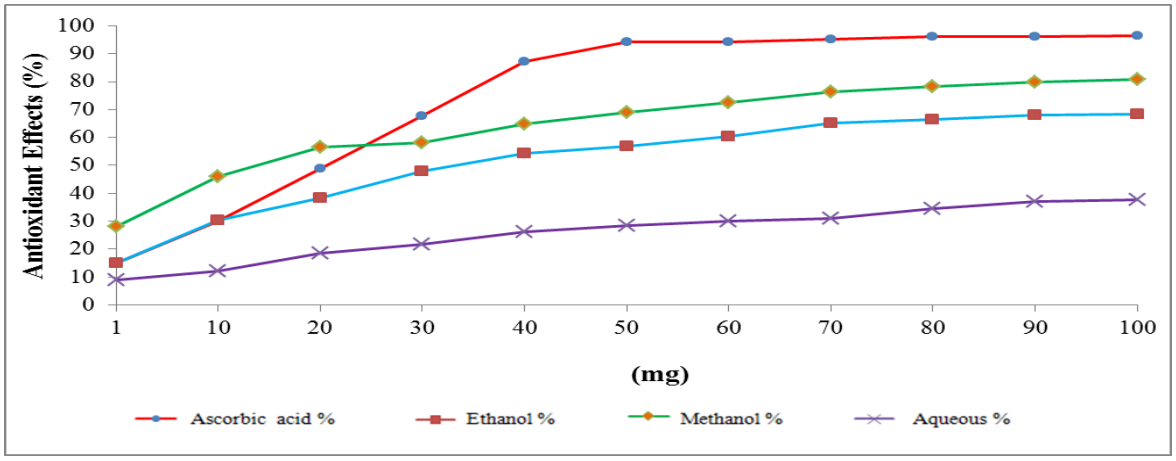


**Fig. 4 (b): Free radical scavenging activity of Flavonoids extracts of Lantana camera and L-ascorbic acid (control) was spectrophoto metrically measured at 517 nm using the DPPH assay.**

**Plants:** ***Tridax procumbens***
**Alkaloid extract**

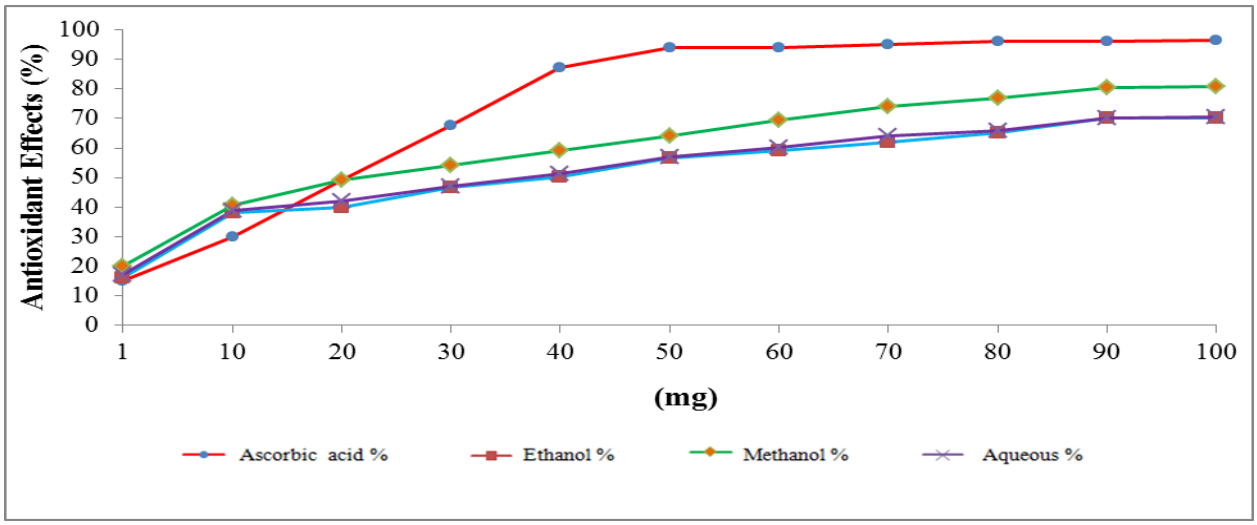


**Fig. 5(a): Free radical scavenging activity of Alkaloids extracts of *Tridax procumbens* and L-ascorbic acid (control) was spectrophotometrically measured at 517 nm using the DPPH Assay.**

**Flavonoid extract**

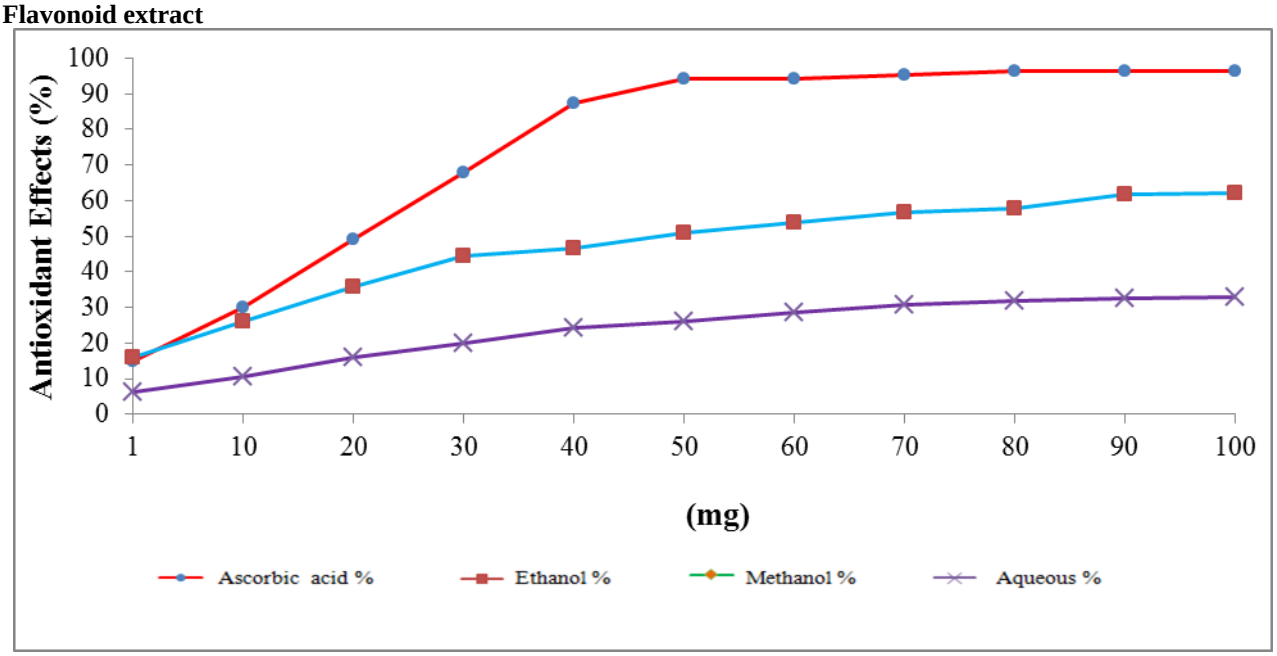


**Fig. 5(b): Free radical scavenging activity of Flavonoids extracts of Tridax procumbens and L-ascorbic acid (control) was spectrophoto metrically measured at 517 nm using the DPPH assay.**

## DISCUSSION

Antioxidant or free radical scavenging activity of extracts from these selected medicinal plants prepared in different solvents was investigated adopting DPPH model system. This study indicated that alkaloid and flavonoidfractions of the plant leaves of *A. indicum A. visca*, *D. stramonium*, *L. camara* and *T. procumbens*, are endowed with antioxidant potential. Further the flavonoid fraction of the extract showed highest antioxidant activity than the alkaloidfraction of the extracts.

The results from the study show that flavonoid extract of *A. visca* (85.10%) and *L. camara* (80.90%) have high potential as antioxidants. The second highest antioxidant activity was observed with *A. indicum* (81.07%), followed by *D stramonium* (75.46). *T. procumbens* extracts showed least activity (62.07%).

Our results compare with other medicinal plants; the ethanolicextracts of *Ocimumbasillicum* leafranked first with an inhibition of free radical scavenging (96.18%). This is followed by *Alpiniacalcarata* leaf (94.63 %). The plant extracts namely *Verbascumthapsus* leaf, *Jatropagossipifolia* leaf, *Jatropamultifida* flower, *Hyptissuaveolens* leaf, *Solanum indicum* leaf,*Clitorria ternate* leaf and flower exhibited similar scavanging effects (Nunez et al,. 2002). The plant extracts showed low radical scavenging effects as seen with etanolic extracts of *Jatrophacurcas* fruit, *Acoruscalamus* leaf, *Strebilisaspera* leaf, *Passifloraedulis* fruit and *Sauropus androgynous* leaf. *Ocimumbasillicum* leaf, *Alpinacalcarata leaf*, *Jatropamultifida* flower, *Hyptissuaveolens* leaf, *Solanum indicum* leaf and *Clitorria ternate* leaf & flower possessed higher radical scavenging activity in both the solvent systems. The antioxidant capacity of plant extract may be due to the hydrogen donating ability of phenols and flavonoids present in it (Nithya & Balakrishnan 2011).

In the present study among the three solvent extracts tested, the methanolic extracts exhibited maximum radical scavenging effect, followed by ethanol and aqueous extracts. The present investigation suggests that medicinal plants which possess good antioxidant potential are the best food supplements for the diseases associated with oxidative stress.

## CONCLUSION

This study reveals that tested plant materials from *A.indicum, A. visca*, *D. stramonium, L.camara* and *T.procumbens,* exhibitmoderate to significant antioxidant activity and free radical scavenging activity. The result of the present study suggests that selected plants can be used as a source of natural antioxidants for pharmacological preparations.

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