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Evolution of free radical scavenging activity of *Luffa acutangula* Linn. Var. *amara*. Roxb. Fruit extract

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Abstract
Natural products are always helpful in the maintenance of life and good health. L. amara is an important wild medicinal plant belongs to the family Cucurbitaceae. All parts of the plant are used to cure many diseases, especially in crystalline bitter compound with cucurbitacin B and Amarinin. The whole plant is used to cure many diseases such as Anticancer, Antidiabetic, Antijaundice and many ailments. The aim of the present study was to evaluate the wild variety of L. amara fruit extract of antioxidant activity by DPPH, H_2O_2 & Super oxide. The fruit extract showed potent antioxidant activity i,e., the overall antioxidant activity of the fruits extract might be attributed to the presence of secondary metabolites. Among the different extracts of fruits of L. amara were used, ethanol, Petroleum Ether, Chloroform and Water. The methanolic extracts of fruits of (80µg/ml) showed higher antioxidant activity than the other solvents extracts used to compare to ascorbic acid. The above results conclude that the ethanolic extracts of L. amara can be used as a natural antioxidant activity.

Keywords: *Luffa acutangula Linn. Var. amara. Roxb, Cucurbitaceae, antioxidant activity, Dpph, H_2o_2, Super oxide*

Introduction
Plants have a key role in the human health care. About 80% of the world population relies on the use of traditional medicine which is predominantly based on the plant materials (Kumar *et al*., 2011) [13]. India has the long history of using plants as medicines in the world. They are estimated to be around 25,000 effective plant-based formulations, used in folk medicine and known to rural communities in India (Verma and Sing, 2008). Various phytoconstituents are obtained from various parts of plant in bark, leaves, flowers, roots, fruits, seeds, etc. may contain the active phytoconstituents. Secondary products from plants have maximum medicinal effects (Meena and Patni, 2008) [17]. The constituents obtained from plants have many pharmacological activities like anti-oxidant, anti-cancer, anti-diabetic, anti-bacterial, anti-viral, anti-ulcer (Farhan *et al.*, 2012) [8] activities. Antioxidant activity is a fundamental as well as important property for human life.
A free radical is a compound with one or more unpaired electrons in its outer orbital (Jasberger 1991) [10]. Such unpaired electrons make these species very unstable and therefore quite reactive with other molecules. Antioxidant compounds in food play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. Some compounds, such as gallates have strong antioxidant activity, while others, such as the mono-phenols are weak antioxidants.
A very recent report shows that the water extracts from fresh sponge gourds exhibited over 80% inhibition. No generation stimulated by lipopolysaccharide, an over 40% inhibitory effect on DNA damage induced by SNP in RAW 264.7 macrophage and a 17.9% scavenging effect on 1,1-diphenyl-2-picrylhydrazylradical (DPPH) radical, as BHA gave a 94.8% scavenging effect (Bor *et al.*, 2006) [4]. In addition, the fresh fruits of *L. amara*, another species showed a certain antioxidant activity (Ansari *et al* 2005) [2]. Previous studies of chemical constituents of this plant mainly involved triterpenoids saponins, although apigenin, a flavone glycoside was isolated from the leaves (Liang *et al.*, 1996, Takemoto *et al.*, 1984, Xiong *et al.*, 1994) [15, 22, 27]. The present study focused on the screening of water-soluble antioxidant compounds based on isolation procedures using high-speed counter current chromatography (HSCCC) (Du *et al.*, 2004 & 2005) [6 -7]
, guided by DPPH radical scavenging activity assay. The results yielded eight antioxidant phenolic compounds, which have not yet been reported in the fruit of *L. amara.* The aim of the present study was to analyze various chemical compounds and free radical scavenging antioxidant activities in the various extracts of *Luffa acutangula* to evaluate their influence of the blood glucose level of normal rats under starch induced antioxidant activity. This research was also providing an electrophoretic protein as a quality control tool for standardization.

Materials and Methods
Chemicals
2, 2 – diphenyl – 1 – Picrylhydrazyl (DPPH), nitro blue tetrazolium (NBT), ferric chloride ($FeCl_3$), ferricyanide,

hydrogen peroxide (H_2O_2), Ascorbic acid were obtained from Sigma-Aldrich chemicals (Mumbai).

Plant Materials

The wild variety of *Luffa acutangula* Linn. Var. *amara*. Roxb. Fruit were collected from the experimental garden in the Department of Plant Science, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India. The leaves and Fruit were dried under shade in room temperature, segregated, pulverized by mechanical grinder and passed through a 40-mesh sieve.

DPPH - (2, 2 - Diphenyl -1, 1, Picryl Hydrazyl) Radical Scavenging Activity

The free radical scavenging capacity of the fruit extracts of *Luffa acutangula* Linn. Var. amara. Roxb was determined using DPPH method (Annie Shirwaiker *et al.,* 2006) [1]. DPPH solution 0.1 mM was prepared in 95 % methanol and 1 ml of this solution was added to 0.3 ml of sample in different concentrations of (20 µg, 40 µg, 60 µg, and 80 µg/mL). The reaction mixture was shaken well and incubated for 30 minutes at dark condition at room temperature after that absorbance was measured at 517nm using UV/Vis spectrophotometer. Ascorbic acid was used as standard at different concentrations of (20 µg, 40 µg, 60 µg, and 80 µg/mL) and a control was prepared without adding extract. The experiment was repeated again and the DPPH scavenging activity was calculated by

Where: $\frac{A_0 = \text{Absorbance of the control}}{A_1 = \text{Absorbance of the sample solution}}$ $[A_0=A_1/A_0] \times 100$

Hydroxyl Radical Scavenging Activity

Hydroxyl radical scavenging activity was measured by the salicylic acid method (Smirnoff and Cumbes, 1989) [21] with some modifications. The extracts at different concentrations of (20µg, 40µg, 60µg, and 80µg) was dissolved in 1 ml of distilled water. 1ml of extracts was mixed with 1 mL of 9mM salicylic acid, 1 ml of 9mM ferrous sulphate and 1 ml of 9 mM hydrogen peroxide. The reaction mixture was incubated for 60 min at 37°c in a water bath after incubation the absorbance mixtures was measured at 510 nm using a UV/Vis spectrophotometer. Ascorbic acid was used as standard at different concentrations of (20µg, 40µg, 60µg, and 80µg/mL) and a control was prepared without adding extract. The experiment was repeated again and the Hydroxyl scavenging activity was calculated by the formula

Where: $\frac{A_0 = \text{Absorbance of the control}}{A_1 = \text{Absorbance of the sample solution}}$ $[A_0=A_1/A_0] \times 100$

Super oxide scavenging activity by alkaline DMSO method

Super oxide scavenging activity is generated by the addition of sodium hydrochloride air saturated DMSO method (Kunchandy and Rao, 1990) [14] with some modifications. The reaction mixture was containing 0.1 ml of Nitro blue tetrazolium (1mg/mL), 0.3 ml of extract in different concentrations of (20µg,40µg,60µg,80µg/mL) and l ml of alkaline DMSO (1ml of alkaline DMSO containing sodium hydrochloride 5mM in 0.1 ml of water) to prepare final volume of 1.4 ml. The absorbance was measured 560 nm using a UV/Vis spectrophotometer. Ascorbic acid was used standard at different concentrations of (20µg, 40µg, 60µg, and 80µg/mL) and a control was prepared without adding extract. The experiment was repeated again and the Super oxide scavenging activity was calculated by

Where: $\frac{A_0 = \text{Absorbance of the control}}{A_1 = \text{Absorbance of the sample solution}}$ $[A_0=A_1/A_0] \times 100$

Results and Discussion

DPPH Radical Scavenging Activity

The Scavenging ability of fruit extracts of *Luffa acutangula* Linn. *Var. amara.* Roxb was shown in (fig.1). The antioxidant efficacy of *Luffa* fruit extracts was quantified spectrophotometrically by changing the DPPH colour from purple to yellow colour. The DPPH was considered to be a model of lipophilic radical. A chain reaction in lipophilic radicals was initiated by lipid auto oxidation. Being a stable free radical, DPPH is regularly used to determine radical scavenging activity of natural compounds. In its radical form, DPPH absorbs at 517 nm, and its absorbance decreases upon reduction with an antioxidant (Marsden and Blois (1958) [16]. Among the different extracts of fruits of *Luffa acutangula* Linn. Var. *amara*. Roxb methanolic extracts fruits (80µg/ml) showed higher antioxidant activity than the other solvents extracts used. The result of DPPH scavenging activity assay in this study indicated that the plant was potently active. This suggested that the plant extract contained compounds that were capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity. The scavenging activity of DPPH radical by the plant extract was found to be appreciable; this implied that the plant extract might be useful for treating radical related pathological damages especially at higher concentration. (Wang *et al.,* 1998) [25]The DPPH radicals enabling the determination of antioxidant capacity of both hydrophilic and lipophilic compounds. (Magalhaes *et al*., 2008) [18]. *In vitro* antioxidant activity of extract was investigated by DPPH (1-1 diphenyl-2-picrylhydrazyl) scavenging activity and by superoxide free radical scavenging activity. To elevate antiradical activity of water extract of *Luffa acutangula* fruit exhibited DPPH radical scavenging activity in concentration dependent manner. This method is based on the reduction of methanolic DPPH solution in the presence of hydrogen donating antioxidant (AH) due to the formation of non-radical form DPPH-H by the reaction DPPH+AH = DPPH-H +A. The remaining DPPH measured after a certain time, corresponds inversely to the radical scavenging activity of the antioxidant. (Tripathi *et al*., 1996) [23]. The sensitivity of the method is determined by the strong absorption of DPPH. In the present study the information on the reactivity of test compounds with a stable free radical since the electron of DPPH give strong absorption band at 517 nm and when it is quenched by the extract there is a decreased in absorbance. Water extract of *Luffa acutangula* fruit showed good antiradical activity in scavenging DPPH radical with IC_{50} (µg/ml) was compared with standard ascorbic acid value.

Hydroxyl Radical Scavenging Activity

The Scavenging ability of *Luffa acutangula* Linn. Var. *amara*. Roxb was measured through Hydroxyl radical scavenging assay as shown in (Fig.2). The radical scavenging capacity of the sample might be attributed to

phenolic compounds in the sample. In this present study the percentage inhibitions were increased with increasing concentrations of the inhibiting antioxidants activity. Among the different extracts of fruits of *Luffa acutangula* Linn. Var. *amara.* Roxb were used chloroform extracts of fruits (80µg/ml) showed higher antioxidant activity than the other solvents extracts used. Hydroxyl radical is highly reactive oxygen centered radical formed from the reaction of various hydro peroxides with transition metal ions. It attacks proteins, DNA, polyunsaturated fatty acid in membranes and most biological molecules it contacts (Aruoma 1999) [3]
and is known to be capable of abstracting hydrogen atoms from membrane lipids (Yen and Duh 1994) [26] that brings about peroxidation reaction of lipids. In the present study a significant correlation existed between the concentration and hydroxyl radical scavenging ability of extract.

Super Oxide Scavenging Activity by Alkaline DMSO Method

The scavenging ability of inhibiting antioxidants activity was further confirmed by super oxide assay. Super oxide (0_2^-) radicals easily react with DNA and protein which necessitate their immediate clearance in living systems. Fluorescent light illumination of serum bovine milk was generated superoxide anion (Karycka-Dahl and Richardson, 1978) [12]. Super oxide radicals are known to be very harmful to the cellular component. Super oxide free radical was formed by alkaline DMSO which reacts with NBT to produce coloured diformazon. Among the different extracts of fruits of *Luffa acutangula* Lin. var. *amara*. Roxb were used, methanol extracts of fruits of (80µg/ml) showed higher antioxidant activity than the other solvents extracts used. The results are comparable with early reports, potent antioxidant activity of *Luffa acutangula* fruits extracts was observed using different methods. However, the efficacy of extract to scavenge the different radicals differed in each method depending upon the involved mechanism of free radical scavenging and adopted assay methodology. Superoxide anion is an oxygen-centered radical with selective reactivity. This species is produced by a number of enzyme systems in auto-oxidation reactions and by non-enzymatic electron transfers. Its univalent function reduces molecular oxygen. It can also reduce certain iron complexes such as cytochrome. (Gulcin *et al.,* 2005) [9].The present study showed potent superoxide radical scavenging activity of *L. cylindrica* leaf extract. The scavenging activity of such radical by the plant extract when compared favorably with the standard reagents such as ascorbic acid suggested that the plant might also serve as a potent scavenger of superoxide radical. Superoxide radicals are very harmful cellular component, can cause tissue or DNA damage leads to various diseases, therefore it always recommended to measure comparative interceptive ability of antioxidant extract to scavenge superoxide molecule. (Vani *et al*., 1997) [24]. The phosphor molybdenum assay is a quantitative method to evaluate water-soluble and fat-soluble antioxidant capacity (total antioxidant capacity), in which transforming of relative free radical species MO (VI) into more stable MO (V) non-reactive products occurs. (Jayaprakasha *et al*., 2008). The ethanol extract of LAP showed highest reducing ability by chelating ferrous ion effectively. The ethanol extract was found to be most efficacious in all *in vitro* assays; it may be due to presence of phenolics in this extract. (Povichit *et al*., 2010) [19].

Conclusion

The results obtained in the present investigation indicated that the methanolic extracts of fruits of *L. acutangula* exhibited free radical scavenging activity. The overall antioxidant activity of the fruits extract might be attributed to the presence of secondary metabolites. The findings of the present study suggested that *L. acutangula* fruits could be a potential source of natural antioxidants having great importance as therapeutic agent in preventing or slowing the oxidative stress related degenerative diseases.

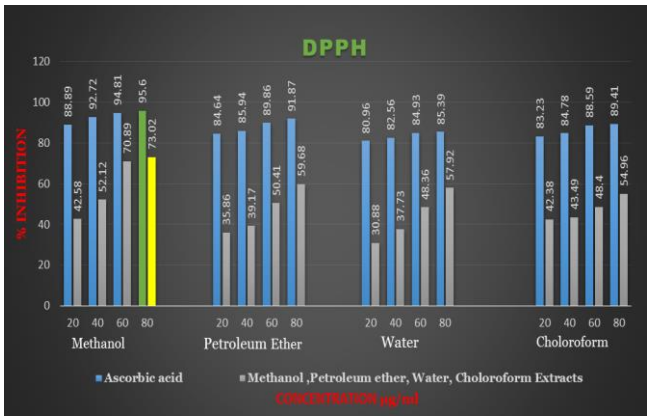

Fig 1: DPPH Free Radical Scavenging Activity of Different Extracts

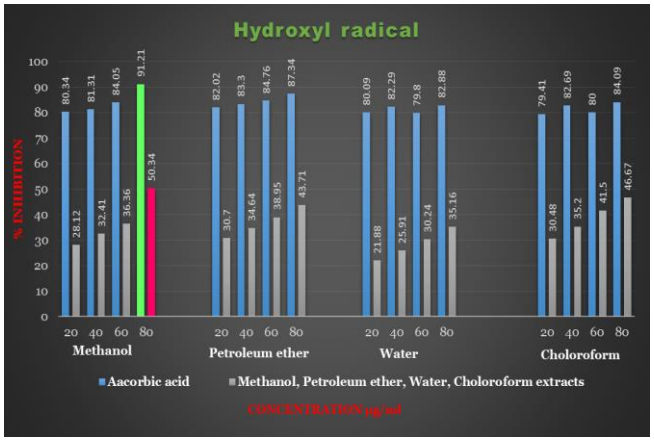

Fig 2: Hydroxyl Radical Scavenging Activity of Different Extracts

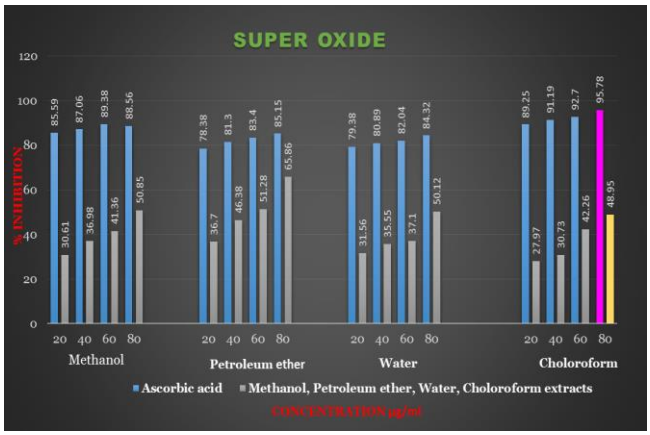

Fig 3: Super Oxide Radical Scavenging Activity of Different Extracts

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