Research Article

Assessment of Potential Antioxidant Activity of Polyphenolic Fraction Separated from Acalypha Indica Leaves: An In vitro Approach

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ABSTRACT

The free radicals are generated in normal cellular metabolic events .However they may be produced more in disease conditions when they directly interact with the bio molecules are dangerous to the normal life because they alter genetic material. Free radicals induce permanent modifications in genetic material that may lead to mutagenesis, carcinogenesis and aging. The prevention or treatment of diseases has direct link with antioxidants which can reduce the effect of free radicals. The phenolic compounds are functionally potent antioxidants and they have the ability to scavenge reactive oxygen species (ROS)/ reactive nitrogen species(RNS), suppress the ROS/RNS formation by inhibiting relevant enzymes, chelates trace metal elements responsible for free radical generation and up regulates or protects the antioxidant defense system. In this study, we attempted for the separation of Polyphenolic fraction from Acalypha indica leaves and tested for its antioxidant activity employing DPPH, H2O2 assays and also to assess its reducing power, total antioxidant power. The outcome of the results from this study showed that Polyphenolic fraction from the Acalypha indica leaves scavenges the in vitro free radicals to their stable forms such as DPPH radical to DPPH, Mo (VI) to Mo (V), ferric cyanide complex to ferrous form and hydrogen peroxide to water. The polyphenolic fraction has the reduction power against free radicals which suggests further studies to take up in in vivo for the treatment of various diseases due to free radicals generation.

Keywords: Acalypha indica, antioxidants, DPPH, free Radicals, reducing Power

Received 30 July 2015 Received in revised form 13 August 2015 Accepted 15 August2015

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INTRODUCTION

The living aerobic organisms developed antioxidant defense system in their body to protect from free radicals generated during biological events in cell. Excess of free radicals are generated in the body when normal system is altered. Alteration in biochemical events in the body leads to diseases such as Cancer, Diabetes, Hepatitis, Alzheimer etc. Under the disease condition, the antioxidant defense system of the body may not be enough to work against free radicals generated, so complementary antioxidants would be/ required supplemented from outside in the form of natural antioxidant rich food substances to decrease the effect of free radicals. Natural antioxidants in food substances are present in the form of phenolic structures and flavanoid structures [1].

The usage of medicinal plants to cure human diseases has long history with Ayurveda. In Ayurvedic medicine a particular plant or combination of plants are used to treat many diseases without having enough knowledge about particular target that is responsible for the causing disease and the mechanism of action of plant crudes. Now a day the scientists are exploring the clues for the causative factors of the diseases and also the mode of action of Ayurvedic medicine. Hence medicinal plants have been occupied very important role in the research of pharmaceutical science. Synthetic drugs for various diseases

are showing adverse effects, the same has been minimized by the scientists using medicinal plants which contain chemical bioactive compounds such as Polyphenols, Alkaloids, Terpenoids, Saponins etc.,

Acalypha indica is a weed plant that occurs throughout the plane of India, China and South Africa. It belongs to the family Euphorbiaceae. *Acalypha indica* has been used traditionally for treating many diseases such as Post-coita, Infertility, Anti-venom, Wound healing, Antioxidant, Inflammation, Diuretic effects, Bacterial infections and Cancer [2-8]. Preliminary phytochemical screening of *Acalypha indica* leaves (**Figure 1**) shows that it has Polyphenols, Flavonoids, Alkaloids, Saponins, Terpenoids and Tannins [9].

Figure 1: *Acalypha indica* plant and its leaves

The phenolic compounds obtained from the plant are functionally potent antioxidants and they have the ability to scavenge reactive oxygen species (ROS)/ reactive nitrogen species(RNS), suppresses the ROS/RNS formation by inhibiting relevant enzymes, chelates trace metal elements responsible for free radical generation and up regulates or protects the antioxidant defense system [10]. Due to having potent antioxidant activity these phenolic compounds made a remark in the suppression of oxidative stresses condition that is associated with diseases [11]. So, the separated polyphenols from various plants could do better work against oxidative stress under diseased condition. In the present study, we attempted to separate the polyphenolic fraction from *Acalypha* leaves using standard protocols and tested for its *in vitro* antioxidant activity.

MATERIAL AND METHODS

Separation of polyphenolic fraction

The separation of polyphenolic fraction from medicinal plants as described by Paulo et al., [12] was followed in this study .The separation of polyphenolic fraction from *Acalypha indica* leaves restricted to three step process.1) the plant leaves contained hydrophobic compounds were removed by the dichloromethane (DCM). The leaf powder was soaked in DCM for overnight with occasional shaking. The DCM extracted compounds were filtered with Whatman No.1 filter paper. The residue again extracted with DCM and filtered, the remaining residue was used for polyphenolic fraction separation. 2) Residue obtained from the first step was extracted with Methanol/Water (70:30 v/v) for overnight soaking and occasional shaking filtered it and again the residue was extracted with the same solvent so that the organic phase (methanol) in the filterate was removed with rotary evaporator at room temperature. The concentrated filterate (aqueous without methanol) contained polyphenolic fraction was separated in the next step. 3) The filtrate (aqueous) was adjusted to pH 4 with 1N HCl. 5 gm of poly vinyl poly pyrrolidine (PVPP) (30 mg/ml) was added to filterate with vigorous shaking for 15 minutes. The PVPP adsorbed polyphenols was separated with Whatman No.1 filter paper, the filtered filterate again treated with PVPP and once again filtered. The PVPP adsorbed polyphenols residue on filter paper was extracted with acetone/water (70:30 v/v) for two times and filtered it with Whatman No.1 filter paper and mixed with filtrate. The filterate that contained polyphenols was concentrated in rotary evaporator, stored for further *in vitro* assays.

Assessment of free radical scavenging activity of polyphenolic fraction

(1) DPPH Assay

The free radical scavenging activity of polyphenolic fraction obtained from *Acalypha* leaves was measured by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) method as described by Shimada et al., [13]. 0.1 mM solution of DPPH was prepared in methanol. To 3 ml of various concentrations of polyphenolic fraction (100 µg/ml to 500

µg/ml), added 1 ml of DPPH solution. The tubes were shaken and incubated in dark for 30 minutes. The stable reaction mixture absorbance was measured at 517 nm using UV-Visible Spectrophotometer. Decreased absorbance indicates increased free radical activity of plant polyphenolic fraction. The percent change was calculated using equation as follows:

DPPH. scavenging activity (%) = $[(A_0-A_1)/A_0] \times 100$

where A_0 was the absorbance of the control and A_1 was the absorbance of the sample or standard.

(2) Total antioxidant activity:

The total antioxidant activity of polyphenolic fraction obtained from acalypha leaves was evaluated by the method described by Prieto et al., [14]. In brief, 0.3 ml of various concentrations (200 to 1000 µg/ml) of sample solution was mixed with 3 ml of reagent solution (contained 0.6 M H_2SO_4, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes were incubated in water bath at 95°C for 90 minutes. Tubes were removed from water bath and allowed to cool. The absorbance of total reaction was read at 695 nm against blank. Blank contained same quantities of reaction mixture but instead of plant sample respective solvent (water) was used. Ascorbic acid was used as a standard. Increased in absorbance indicates increased percentage of total antioxidant activity of polyphenolic fraction.

(3) Reducing power

Fe3+ ion reducing power determined in the polyphenolic fraction obtained from *Acalypha Indica* leaves using the method described by Mohammad Ali et al., [15]. To 0.5 ml of various concentrations of polyphenolic fraction (100 to 500 µg/ml), added 0.75 ml of phosphate buffer (0.2 M, pH 6.6) and 0.75 ml potassium hexaferricyanide (1% w/v). The reaction mixture was incubated for 20 min at 50 °C in water bath. The reaction was arrested by adding 0.75 ml of trichloroacetic acid (TCA) (10% w/v). Then centrifuged it at 3000 rpm for 10 minutes. 1.5 ml of supernatant was collected and mixed with the same amount of distilled water plus 0.1 ml $FeCl_3$ (0.1% w/v) and allowed for 10 minutes incubation at room temperature. The reducing power of polyphenolic fraction was recorded at 700 nm in UV Vis spectrophotometer. Ascorbic acid was used as standard.

(4) Hydrogen peroxide scavenging assay

Mohammad Ali et al., method [15] was used to determine the hydrogen peroxide scavenging activity of polyphenolic fraction obtained from *Acalypha* leaves. Hydrogen peroxide (10 mM) solution was prepared in phosphate buffer (50 mM, pH 7.4). To 1 ml of various concentrations of plant polyphenolic fraction (200 to 1000 µg/ml) added 2 ml of hydrogen peroxide and incubated it for 30 minutes at room temperature. Unreacted hydrogen peroxide was measured at 230 nm against phosphate buffer blank. Ascorbic acid was used as a standard. The percentage of hydrogen peroxide scavenging activity was calculated using the following formula

(1-absorbance of sample/absorbance of control) x100.

RESULTS AND DISCUSSION

The plants have rich amount of phenolic and flavanoid compounds as their secondary metabolites. Plant secondary metabolites have antioxidant ability due to hydrogen or electron donation to adjacent oxidised compounds [16]. The antioxidant rich parts of the plant are used for treating of many diseases due to their antioxidant property. The disease influences the production of many reactive oxygen species which are unstable and react with the bio molecules to damage the normal function of cell [17]. Supplementation of plants bio active compounds contained antioxidants reduce the reactive oxygen species under disease condition and reduce the over load on normal antioxidant defence system.

$DPPH^{\cdot}$ is generally used as free radical scavenging for identification of antioxidant activity of plant extract sources. Plant sources contain many antioxidants which donate hydrogen to $DPPH^{\cdot}$ radical to stable it in its reduced form [1]. The amount of $DPPH^{\cdot}$ radical reduced determines the scavenging activity of plant extract. The (**Figure 2**) shows that the polyphenols from *Acalypha indica* reduced the $DPPH^{\cdot}$ radical in concentration manner and its IC50 value was calculated. Increased concentration of polyphenolic fraction, increased reduction

of DPPH· radicals and its 50% free radical reduction capacity has been observed at 300.588 µg/ml.

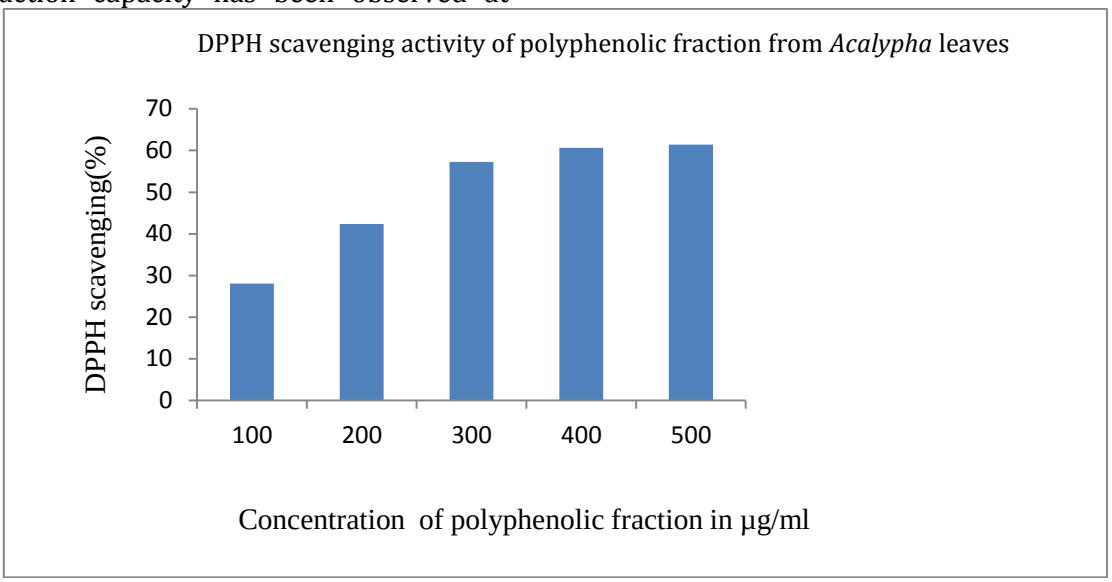

Figure 2: DPPH free radical scavenging activity (%) at different concentrations of poly phenolic fraction obtained from *Acalypha indica*.

The principle mechanism involved in total antioxidant activity was; reduction of Mo (VI) to Mo (V) in the presence of antioxidant compounds present in the bioactive compounds sample. The reduced form of Mo (V) as phosphate/Mo (V) green color complex is due to polyphenols in *Acalypha indica* and the same was measured at 695 nm compared with standard Ascorbic acid. The graph (**Figure 3**) clearly shows that the antioxidant activity was depend on the concentration manner but its reduction rate move constantly after fourth concentration. The fourth concentration (800µg/ml) contained antioxidants enough for conversion of Mo (VI) to Mo (V). The absorbance of polyphenolic fraction and the standard Ascorbic acid have more equal capacity for the formation of phosphate/Mo (V) green color complex so that the polyphenols have helpful characteristic of antioxidant capacity.

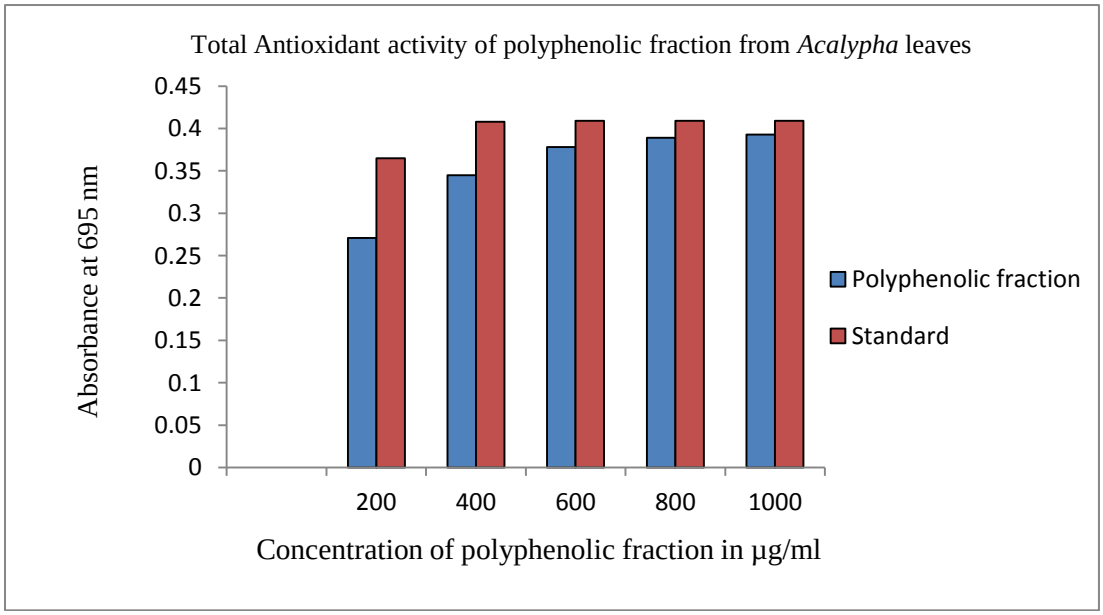

Figure 3: Total antioxidant activity (Absorbance at 695 nm) at different concentrations of polyphenolic fraction obtained from Acalypha indica.

Antioxidants act as reducing agents [18]. Reducing power of plant compounds (antioxidants) present in the test samples determines the conversion of ferric cyanide complex to ferrous form. The formation of Pearl's Prussian blue can be examined by measuring the color intension at 700 nm in UV-VIS spectrophotometer. Increased concentration increases the absorbance of color [19]. The polyphenolic fraction of *Acalypha indica* showed reducing activity against ferric cyanide complex (**Figure 4**). Plant Polyphenolic fraction and the standard in a concentration manner revealed that plant polyphenolic fraction have lower reducing activity than standard ascorbic acid.

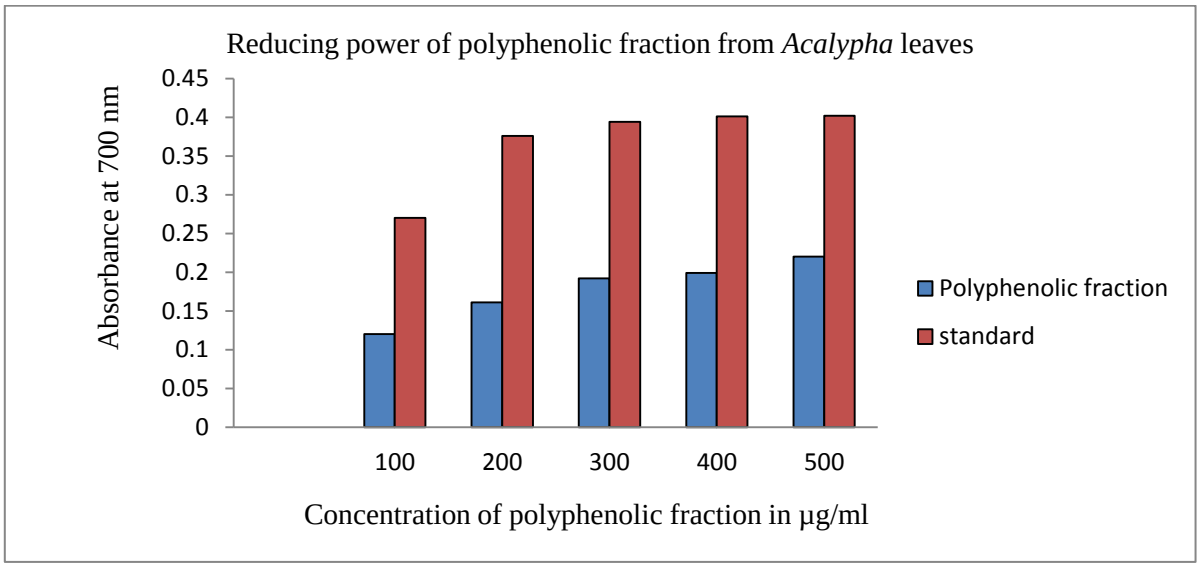

Figure 4: Reducing power (absorbance at 700nm) at different concentrations of polyphenolic fraction from *Acalypha indica*

Hydrogen peroxide is a non-reactive substance in cell but its higher concentration leads to toxic condition because it changed to hydroxyl radicals [20]. These hydroxyl radicals interact with the bio molecules leads to many diseases. Because of adverse effects on bio molecules, the hydrogen peroxide formed must be minimized in the cell. Polyphenolic fraction obtained from *Acalypha indica* leaves has the efficacy in scavenging free radicals generated from hydrogen peroxide based on concentration manner (**Figure 5**). Increase in concentration leads increase in scavenging activity of free radicals. The half hydrogen peroxide scavenging concentration of polyphenolic fraction was noticed at the concentration of 703.88 µg/ml.

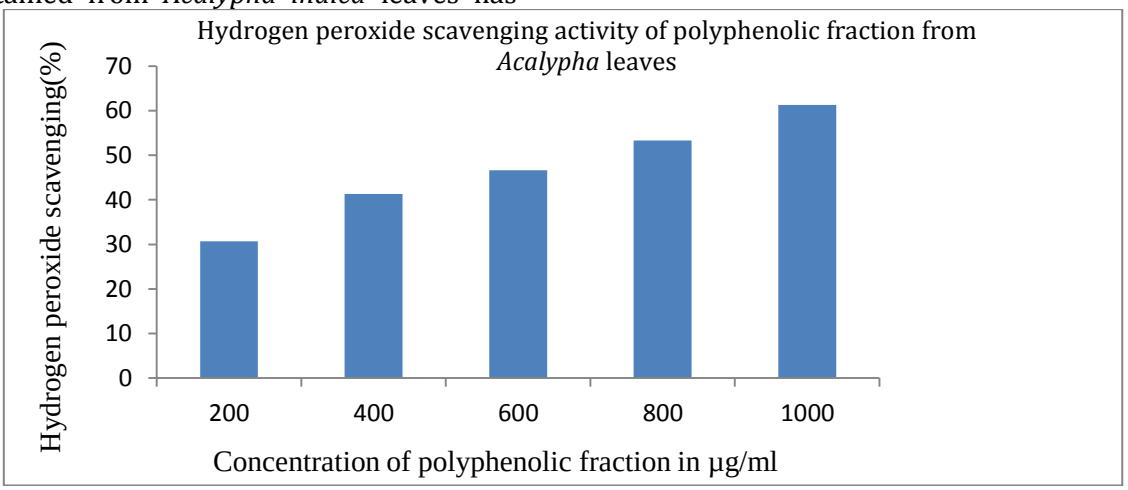

Figure 5: Hydrogen peroxide scavenging activity (%) at different concentrations of poly phenolic fraction from Acalypha indica

CONCLUSION

Polyphenolic fraction obtained from *Acalypha indica* leaves has free radicals scavenging activity since DPPH radical was reduced to DPPH, Mo (VI) to Mo (V), ferric cyanide complex to ferrous form and hydrogen peroxide to water. The polyphenolic fraction obtained from *Acalypha indica* leaves exhibits free radical scavenging activity when tested for *in vitro.* Hence these polyphenolic fraction obtained may be subjected for further preclinical study by *in vivo* method under diseased conditions to prove its antioxidant activity.

ACKNOWLEDGEMENT

The first author is grateful to the University Grants Commission New Delhi, India for their financial assistance through RGNF (Rajiv Gandhi National Fellowship-No: F1-17.1/2012-13/R GNF-2012-13-SC -AND-34335), and also UGC-BSR- One Time Grant (No: F.19-141/2014 (BSR) dated 16-12-2014) sanctioned to the corresponding author.

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