World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

In vitro antioxidant potential of secondary and tertiary growth forms of *Salvinia molesta* Mitchell

Gini Thottappilly George *, Jeya Jothi Gabriel

Department of Plant Biology and Biotechnology, Loyola College, Nungambakkam, Chennai – 600 034, Tamil Nadu, India

Received: 01-02-2016 / Revised: 23-02-2016 / Accepted: 24-02-2016 / Published: 28-02-2016

ABSTRACT

The aquatic weed *Salvinia molesta* causes adverse ecological disruptions leading to considerable social, ecological and economic damages. It reduces dissolved oxygen content, blocks sunlight reaching the other aquatic species and degrades water quality. The present study was undertaken to evaluate the medicinal properties in particular the antioxidant activity of the plant. Hexane, ethyl acetate, ethanol and methanol extracts of secondary and tertiary growth forms of *Salvinia molesta* were studied for 1, 1-Diphenyl-2-picrylhydrazyl, hydroxyl, hydrogen peroxide and superoxide anion free radicals scavenging activities. All the four assays showed that the ethanol and methanol extracts of both growth forms were found to have good free radical scavenging activity. The results indicated that this aquatic weed is a potent natural antioxidative therapeutic agent in curing oxidative stress related diseases. Utilization of the plant for pharmaceutical applications involving the isolation and identification of useful phytocomponents responsible for its antioxidant activity would help to control its spread as an invasive species.

Key words: Environmentally hazardous, natural antioxidant, oxidative stress, free radical scavenging, phytocompounds, human health

INTRODUCTION

Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with novel remedies [1]. In recent years much attention has been paid to natural antioxidants and their association with health benefits [2]. Plants are potential sources of natural antioxidants to sabotage reactive oxygen species [3]. They possess a wide variety of free radical scavenging molecules such as phenolic compounds, alkaloids, terpenoids, vitamins and some other endogenous metabolites, which are rich in antioxidant activity [4-9]. Antioxidants guard cells from tissue injury and cellular damage caused by precarious molecules known as free radicals [10]. Though radicals are less stable, their reactivity is stronger than the non- radical species [11].

Reactive oxygen species (ROS) and Reactive nitrogen species (RNS) include radicals such as hydroxyl (OH•), superoxide ($O_2^{\bullet-}$), peroxyl ($RO_2^{\bullet}$), alkoxyl (RO•), hydroperoxyl ($HO_2^{\bullet}$), nitrogen dioxide ($NO_2^{\bullet}$), nitric oxide (NO•) and lipid peroxyl (LOO•). Non-radicals include hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), ozone (O_3), singlet oxygen, peroxynitrate ($ONOO^-$), nitrous acid (HNO_2), lipid peroxide (LOOH) and dinitrogen trioxide (N_2O_3) [12].

Physiological and biochemical processes in the human body produce oxygen-centered free radicals and other reactive oxygen species as byproducts. Overproduction of such free radicals cause oxidative damage to biomolecules namely lipids, proteins and DNA, eventually leading to many chronic diseases, such as atherosclerosis, cancer, diabetes, aging and other degenerative diseases in humans [13,14,15]. The occurrence of certain molecules in the human body acting as antioxidants or free radical scavengers can protect the body from oxidative stress related complexities. Antioxidants stabilize free radicals and inhibit the damage caused by these elements [16].

Epidemiological studies have shown that many of the antioxidants possess anti-inflammatory, anti-atherosclerotic, antitumor, anti-mutagenic, anti-

***Corresponding Author Address: Gini Thottappilly George,** Department of Plant Biology and Biotechnology, Loyola College, Nungambakkam, Chennai – 600 034, Tamil Nadu, India; Emaill id: ginigeorgehc@gmail.com*

carcinogenic, antibacterial and antiviral properties [17-20]. The intake of the natural antioxidants has been associated with the reduced risk of cancer, cardiovascular disease, diabetes and other diseases associated with aging, but there is still considerable disputation in this matter [21-25].

Salvinia molesta (*S. molesta*) is a free floating aquatic fern, belongs to family Salviniaceae and is potentially a serious aquatic weed native to Brazil [26]. It is commonly called as giant *Salvinia*, African pyle and Kariba weed [27]. *S. molesta* is a weed of National Significance. It is regarded as one of the worst weeds because of its invasiveness, potential for spread, economic and environmental impacts [28]. Dense mats of giant *Salvinia* interfere with rice cultivation, clog fishing nets and disrupt access to water for humans, livestock, wildlife, recreation, transportation, irrigation, hydroelectric generation and flood control are also hampered [29, 30]. The primary objective of the study was to assess the medicinal properties in particular the antioxidant potential of *S. molesta* and subsequent development of the plant as one of the pharmaceutical drugs.

MATERIALS AND METHODS

Chemicals and reagents: 1, 1-Diphenyl-2-picrylhydrazyl (DPPH), 2- deoxy-D-ribose and Nitro blue tetrazolium (NBT) were purchased from Sigma chemicals co., USA, Trichloroacetic acid (TCA), Thiobarbituric acid (TBA), Dimethyl sulphoxide (DMSO), Ethylenediaminetetraacetic acid (EDTA), ascorbic acid, gallic acid, riboflavin, Hydrogen peroxide (H2O2), Ferric chloride (FeCl3) and Phosphate buffer (p^H- 6.8, 7.4) were obtained from Hi- media Pvt. Ltd. Mumbai, India.

Collection and identification of *S. molesta:* The secondary and tertiary growth forms of *S. molesta* were collected separately in the month of April 2012 from Kalliyad, Thrissur, Kerala, India. The plant was identified and confirmed taxonomically by Dr. G. Jeya Jothi, Assistant professor, Department of Plant Biology and Biotechnology, Loyola College, Chennai. Voucher specimen of *S. molesta* (LCH – 130) has been preserved in Loyola College Herbarium for reference.

Preparation of *S. molesta* extracts: The whole plant of *S. molesta* secondary and tertiary growth forms were cleansed under running tap water until all the debris were removed. The plant materials were shade dried for three weeks at room temperature and pulverized into fine powder. The powdered plant materials (50 gm) were extracted with 300 ml of hexane, ethyl acetate, ethanol and methanol sequentially using an orbital shaker (120 rpm) at room temperature for 72h by cold percolation method. The extracts were filtered through Whatman No.1 filter paper and concentrated under reduced pressure using rotary evaporator. The dried crude extracts were stored in the refrigerator at 4^0C in amber glass vials for subsequent experiments.

DPPH radical scavenging assay of *S. molesta:* DPPH radical scavenging ability of *S. molesta* secondary and tertiary growth forms extracts were assessed according to the method of Chan et al [31] and Sanchez – Moreno et al [32] with slight modification. DPPH (3.95 mg) was dissolved in 50 ml methanol. It was protected from light by covering it with aluminum foil. 1 ml of methanol solution of DPPH (200 mM) was mixed with different concentrations of the extracts (200-1000 µg/ml). The total volume was made up to 3 ml using ethanol. Test tubes were shaken well and incubated at 37^0C for 30 min. in darkness. The control contained only DPPH solution in methanol. Ascorbic acid was used as standard antioxidant. All samples were analyzed in triplicates. The percentage of decolourization was obtained at 517 nm using UV-VIS spectrophotometer. The reduction capability of DPPH was determined by the decrease in absorbance. The DPPH free radical scavenging activity was calculated using the following formula:

% of DPPH scavenging = $(A_0 - A_1)/ A_0 \times 100$

Where A_0 is the absorbance of control at 30 min. A_1 is the absorbance of test sample at 30 min.

Hydroxyl radical scavenging assay of *S. molesta:* Hydroxyl radical scavenging capacity of *S. molesta* secondary and tertiary growth forms extracts were measured according to the modified method of Halliwell and Gutteridge [33] based on the ability to compete with deoxyribose for hydroxyl radicals. To the varying concentrations of the extracts (200-1000 µg/ml), 200 µl of 2.8 mM 2-deoxy-D-ribose, 400 µl of 0.2 mM ferric chloride, 100 µl of 1 mM EDTA (1:1 v/v), 200 µl of 1 mM H_2O_2 and 200 µl 1mM ascorbic acid were added in 1.2 ml 20 mM phosphate buffer (p^H- 7.4) and was mixed well to form a reaction mixture (Fenton reaction). It was then incubated at 37^0C for 1h. The reaction was terminated by the addition of 1 ml of 2.8% TCA and 0.5 ml of 1% TBA. The mixture was heated at 100^0C for 45 min. to develop a pink color. The absorbance was measured at 532 nm after cooling the mixture. Ascorbic acid was used as standard reference. Distilled water was used in place of test extracts for control. All samples were analyzed in triplicates. The scavenging activity of hydroxyl radical was calculated using the following formula:

% of hydroxyl radical scavenging = $(A_0 – A_1)/ A_0 \times 100$

Where A_0 is the absorbance of control and A_1 is the absorbance of test sample.

Hydrogen peroxide radical scavenging assay of *S. molesta:* Hydrogen peroxide scavenging activity of *S. molesta* secondary and tertiary growth forms extracts were determined according to the method of Nabavi et al [34] with minor changes. Hydrogen peroxide solution (40 Mm) was prepared in 0.1 M phosphate buffer (p^H-7.4). Different concentrations of extracts (200-1000 µg/ml) were added to 0.6 ml of hydrogen peroxide solution (40mM). The absorbance was measured at 230 nm after 10 min. against blank containing phosphate buffer without hydrogen peroxide. All samples were analyzed in triplicates. The percentage of hydrogen peroxide scavenging was calculated using the following formula:

% of hydrogen peroxide scavenging = $(A_0 – A_1)/ A_0 \times 100$

Where A_0 is the absorbance of control and A_1 is the absorbance of test sample.

Superoxide anion scavenging assay of *S. molesta:* Superoxide anion quenching ability of *S. molesta* secondary and tertiary growth forms extracts were checked according to the method of Robak et al [35] and Beauchamp et al [36] with slight changes. 100 µl of riboflavin solution (20 µg), 200 µl of EDTA solution (12 mM), 200 µl of methanol and 100 µl NBT (0.1 mg) solutions were mixed in a test tube and the reaction mixture was diluted up to 3 ml with phosphate buffer (67 mM). The absorbance was measured at 530 nm after exposing to fluorescent light for 20 min. This was taken as control. Different concentrations of extracts and standard were taken and diluted with 100 µl of methanol. To each of these, 100 µl of riboflavin solution (20 µg), 200 µl of EDTA solution (12 mM), 200 µl of methanol and 100 µl NBT (0.1 mg) solutions were mixed in a test tube and the reaction mixture was diluted up to 3 ml with phosphate buffer (67 mM). The absorbance was measured at 530 nm after exposing to fluorescent light for 20 min. using UV-VIS spectrophotometer. The effect of superoxide anion scavenging was calculated using the following formula:

% of superoxide anion scavenging = $(A_0 – A_1)/ A_0 \times 100$

Where A_0 is the absorbance of control and A_1 is the absorbance of test sample.

Statistical analysis: All experimental measurements were carried out in triplicates and were expressed as mean ± SD. The effective concentration of extracts required to scavenge DPPH, hydroxyl, hydrogen peroxide and superoxide anion free radicals by 50 % (IC50 value) was obtained from the plotted graph of scavenging activity against concentrations of the extracts. Results were obtained by computer program Microsoft Excel (2007) and Regression analysis.

RESULTS

DPPH radical scavenging: DPPH is a stable radical and when it reacts with an antioxidant compound which can donate hydrogen, it is reduced to diphenylpicrylhydrazine. DPPH radical scavenging activity assay showed the ability of *S. molesta* extracts to donate hydrogen or to scavenge free radicals. The DPPH radical scavenging effect of *S. molesta* secondary and tertiary growth forms extracts were evaluated and compared with standard ascorbic acid. The percentage (%) inhibition at various concentrations (200-1000 µg/ml) of *S. molesta* extracts and ascorbic acid were calculated. The IC_{50} values obtained from the graph were 780 µg/ml for *S. molesta* secondary form hexane extract (SmSrHE), 525 µg/ml for *S. molesta* secondary form ethyl acetate extract (SmSrEAE), 280 µg/ml for *S. molesta* secondary form ethanol extract (SmSrEE), 965 µg/ml for *S. molesta* secondary form methanol extract (SmSrME), 600 µg/ml for *S. molesta* tertiary form hexane extract (SmTrHE), 880 µg/ml for *S. molesta* tertiary form ethyl acetate extract (SmTrEAE), 860 µg/ml for *S. molesta* tertiary form ethanol extract (SmTrEE), 280 µg/ml for *S. molesta* tertiary form methanol extract (SmTrME) and 820 µg/ml for ascorbic acid (AA) respectively. The results are presented in Table 1 and 2. The graph is shown in Fig. 1 and 2.

Hydroxyl radical scavenging: The hydroxyl radical scavenging effect of *S. molesta* secondary and tertiary growth forms extracts were evaluated and compared with standard ascorbic acid. The percentage (%) inhibition at various concentrations (200-1000 µg/ml) of *S. molesta* extracts and ascorbic acid were calculated. The IC_{50} values obtained from the graph were 760 µg/ml (SmSrHE), 920 µg/ml (SmSrEAE), 740 µg/ml (SmSrEE), 600 µg/ml (SmSrME), 830 µg/ml (SmTrHE), 925 µg/ml (SmTrEAE), 825 µg/ml (SmTrEE), 560 µg/ml (SmTrME) and 400 µg/ml (AA) respectively. The results are presented in Table 3 and 4. The graph is shown in Fig. 3 and 4.

Hydrogen peroxide radical scavenging: The hydrogen peroxide scavenging effect of *S. molesta* secondary and tertiary growth forms extracts were evaluated and compared with standard ascorbic acid. The percentage (%) inhibition at various

concentrations (200-1000 µg/ml) of *S. molesta* extracts and ascorbic acid were calculated. The IC_{50} values obtained from the graph were 380 µg/ml (SmSrHE), 805 µg/ml (SmSrEAE), 340 µg/ml (SmSrEE), 620 µg/ml (SmSrME), 125 µg/ml (SmTrHE), 640 µg/ml (SmTrEAE), 320 µg/ml (SmTrEE), 520 µg/ml (SmTrME) and 445 µg/ml (AA) respectively. The results are presented in Table 5 and 6. The graph is shown in Fig. 5 and 6.

Superoxide anion radical scavenging: The superoxide anion radical scavenging effect of *S. molesta* secondary and tertiary growth forms extracts were evaluated and compared with standard ascorbic acid. The percentage (%) inhibition at various concentrations (200-1000 µg/ml) of *S. molesta* extracts and ascorbic acid were calculated. The IC_{50} values obtained from the graph were 120 µg/ml (SmSrHE), 970 µg/ml (SmSrEAE), 570 µg/ml (SmSrEE), 420 µg/ml (SmSrME), 760 µg/ml (SmTrHE), 920 µg/ml (SmTrEAE), 460 µg/ml (SmTrEE), 775 µg/ml (SmTrME) and 480 µg/ml (AA) respectively. The results are presented in Table 7 and 8. The graph is shown in Fig. 7 and 8.

DISCUSSION

This study was specifically involved with the investigation of antioxidant properties of *S. molesta*. Free radicals play a vital role in a variety of pathological manifestations. Antioxidants fight against many free radicals and protect the human body from many diseases. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defense mechanisms [37].

In this present study antioxidant potential of various extracts of secondary and tertiary growth forms of *S. molesta* were assessed by four different scavenging assays.

DPPH is a kind of stable organic free radical [38]. The DPPH assay has multiple advantages such as good stability, credible sensitivity and feasibility [39]. IC_{50} value is often used to express the quantity or concentration of extracts needed to scavenge 50% of the free radicals. IC50 value is inversely proportional to the scavenging potential of the extracts. Smaller IC50 corresponds to a higher antioxidant activity of the plant extracts [40]. The DPPH assay showed that SmSrEE had the highest DPPH scavenging potency with IC50 value 280 µg/ml among *S. molesta* secondary growth form extracts and SmTrME had the highest DPPH scavenging potency with IC50 value 280 µg/ml among *S. molesta* tertiary growth form extracts. The IC50 values of SmSrEAE (525 µg/ml), SmTrHE (600 µg/ml) SmSrHE (780 µg/ml) were lower than that of standard AA (820 µg/ml), while SmTrEE (860 µg/ml), SmTrEAE (880 µg/ml), SmSrME (965 µg/ml) had IC50 values higher than AA. The IC_{50} values were in the order of SmSrEE< SmSrEAE< SmSrHE< SmSrME for *S. molesta* secondary form extracts and SmTrME< SmTrHE< SmTrEE< SmTrEAE for *S. molesta* tertiary form extracts. This assay revealed that the extracts have proton-donating ability and can scavenge free radicals in dose-dependent manner, acting possibly as primary antioxidants.

The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cell. Hydroxyl radical scavenging ability of an extract is directly related to its antioxidant activity [41]. The hydroxyl radical is the most reactive of the reactive oxygen species and it induces severe damage in adjacent biomolecules [42]. The hydroxyl scavenging activity revealed that, all the extracts of *S. molesta* secondary and tertiary growth forms had IC_{50} values higher than the standard ascorbic acid (400 µg/ml). Among the extracts SmTrME and SmSrME had the lowest IC_{50} value as 560 µg/ml and 600 µg/ml respectively. The IC_{50} values were in the order of SmSrME< SmSrEE< SmSrHE< SmSrEAE for *S. molesta* secondary form extracts and SmTrME< SmTrEE< SmTrHE< SmTrEAE for *S. molesta* tertiary form extracts. Though the hydroxyl scavenging potential was lower than the standard AA, the present study revealed that methanol extracts of both the growth forms have got rather good hydroxyl radical scavenging capacity.

Scavenging of hydrogen peroxide by the extracts may be due to the phenolic compounds present in them, which can donate electrons to H_2O_2, thus converting into H_2O [43]. *S. molesta* extracts were capable of scavenging hydrogen peroxide in a dose-dependent manner. The maximum hydrogen peroxide scavenging activity was observed in SmSrEE (340 µg/ml) and SmSrHE (380 µg/ml) among secondary growth form extracts and SmTrHE (125 µg/ml) and SmTrEE (320 µg/ml) among tertiary growth form extracts respectively. All the other extracts had scavenging activity below the reference compound AA (445 µg/ml). The IC_{50} values were in the order of SmSrEE< SmSrHE< SmSrME< SmSrEAE for *S. molesta* secondary form extracts and SmTrHE< SmTrEE< SmTrME< SmTrEAE for *S. molesta* tertiary form extracts.

Superoxide anion radical is considered to be the major source of reactive oxygen species [44]. Even though superoxide anion is a weak oxidant, it gives rise to powerful and dangerous hydroxyl radicals as well as singlet oxygen both of which contribute to oxidative stress [45]. Superoxide is a highly reactive molecule that reacts with various substances produced through metabolic processes. [46]. Superoxide scavenging ability is mainly due to the presence of flavonoids in the extracts [47].

In the present study the scavenging efficiency of superoxide anion radicals were assessed in comparison with the same dose of ascorbic acid ranging from 200-1000 µg/ml. The IC_{50} values were in the order of SmSrHE< SmSrME< SmSrEE< SmSrEAE for *S. molesta* secondary form extracts and SmTrEE< SmTrHE< SmTrME< SmTrEAE for *S. molesta* tertiary form extracts. The IC_{50} values of SmSrHE (120 µg/ml) and SmSrME (420 µg/ml) were lower than the standard AA (480 µg/ml) and all the other extracts had higher IC_{50} values than the standard AA. When compared to standard AA (480 µg/ml) the superoxide scavenging activity of SmSrHE (120 µg/ml), SmSrME (420 µg/ml), SmTrEE (460 µg/ml) were found to be high.

All the above assays involved in this study revealed that lesser the IC_{50} value for an extract higher the ability to scavenge free radicals. All the four antioxidant assays performed on *S. molesta* secondary and tertiary growth forms showed that the ethanol and methanol extracts exhibited good antioxidant activity when compared to the other extracts. Some of the *S. molesta* extracts had high free radical scavenging activity than the reference compound while the other extracts had low scavenging effect.

CONCLUSION

The present investigation on the antioxidant properties of *S. molesta* revealed that both secondary and tertiary growth forms of *S. molesta* have the presence of many active phytocompounds and could be effective as natural antioxidants. This discovery further involves the isolation and identification of these phytocomponents and subsequent utilization of these plant constituents for medical applications and formulation of drugs for treating oxidative stress related diseases. This is the first account of studying two different growth forms of *S. molesta* for its antioxidant properties. This study also confirmed that *S. molesta* has become an important plant for pharmaceutical applications. Utilization of the plant pharmaceutically would help to prevent its spread as noxious aquatic weed and prevent large social, ecological and economical losses. Further research is in progress to identify and elucidate the structure of the phytocomponents responsible for the free radical scavenging activity of the plant.

ACKNOWLEDGEMENTS

GTG thanks University Grants Commission (F1-17.1/2012-13/MANF-2012-13-CHR-KER-7693) for providing financial assistance. The authors are grateful to Rev. Dr. G. Joseph Anthony Samy S. J., Principal, Loyola College for the encouragement and support. Authors also thank Prof. Antoine Lebel, Head of the Department of Plant Biology and Biotechnology, Loyola College, Chennai, for every help rendered.

Table 1: DPPH scavenging activity of *Salvinia molesta* secondary form extracts

Sample	Concentration (µg/ml)	% Inhibition	IC_{50} (µg/ml)
Hexane extracts	200	9.04 ± 0.003	780
	400	23.95 ± 0.006	
	600	39.43 ± 0.002	
	800	52.68 ± 0.001	
	1000	65.42 ± 0.002	
Ethyl acetate extracts	200	30.63 ± 0.002	525
	400	40.35 ± 0.003	
	600	50.29 ± 0.001	
	800	60.64 ± 0.002	
	1000	68.33 ± 0.000	
Ethanol extracts	200	47.71 ± 0.002	280
	400	54.78 ± 0.001	
	600	55.48 ± 0.000	
	800	63.38 ± 0.001	
	1000	70.85 ± 0.001	
Methanol extracts	200	8.36 ± 0.000	965
	400	18.44 ± 0.000	

	600	21.70 ± 0.001	
	800	42.17 ± 0.002	
	1000	60.18 ± 0.001	
Ascorbic acid	200	22.77 ± 0.000	820
	400	28.97 ± 0.000	
	600	34.96 ± 0.000	
	800	48.09 ± 0.000	
	1000	58.41 ± 0.000	

Each value represents the mean ± SD of triplicate experiments.

Table 2: DPPH scavenging activity of *Salvinia molesta* tertiary form extracts

Sample	**Concentration (µg/ml)**	**% Inhibition**	**IC_{50} (µg/ml)**
Hexane extracts	200	29.09 ± 0.003	600
	400	38.67 ± 0.004	
	600	51.58 ± 0.000	
	800	60.24 ± 0.000	
	1000	68.17 ± 0.001	
Ethyl acetate extracts	200	3.72 ± 0.001	880
	400	21.81 ± 0.001	
	600	30.77 ± 0.002	
	800	46.99 ± 0.002	
	1000	63.06 ± 0.001	
Ethanol extracts	200	5.89 ± 0.004	860
	400	20.45 ± 0.005	
	600	34.60 ± 0.004	
	800	48.29 ± 0.001	
	1000	63.04 ± 0.001	
Methanol extracts	200	48.26 ± 0.001	280
	400	53.88 ± 0.000	
	600	61.00 ± 0.001	
	800	67.98 ± 0.000	
	1000	73.85 ± 0.001	
Ascorbic acid	200	22.77 ± 0.000	820
	400	28.97 ± 0.000	
	600	34.96 ± 0.000	
	800	48.09 ± 0.000	
	1000	58.41 ± 0.000	

Each value represents the mean ± SD of triplicate experiments.

Table 3: Hydroxyl scavenging activity of *Salvinia molesta* secondary form extracts

Sample	**Concentration (µg/ml)**	**% Inhibition**	**IC_{50} (µg/ml)**
Hexane extracts	200	39.69 ± 0.001	760
	400	42.83 ± 0.001	
	600	43.56 ± 0.001	
	800	52.74 ± 0.001	
	1000	59.86 ± 0.001	
Ethyl acetate extracts	200	20.77 ± 0.001	920
	400	25.86 ± 0.002	
	600	27.05 ± 0.002	
	800	34.73 ± 0.002	
	1000	55.99 ± 0.002	
Ethanol extracts	200	24.33 ± 0.001	740
	400	39.06 ± 0.001	
	600	44.92 ± 0.001	

	800	47.57 ± 0.001	
	1000	60.80 ± 0.002	
Methanol extracts	200	37.52 ± 0.002	600
	400	46.46 ± 0.002	
	600	50.09 ± 0.002	
	800	57.00 ± 0.002	
	1000	63.56 ± 0.002	
Ascorbic acid	200	47.07 ± 0.002	400
	400	50.16 ± 0.002	
	600	52.20 ± 0.006	
	800	55.93 ± 0.002	
	1000	62.58 ± 0.001	

Each value represents the mean ± SD of triplicate experiments.

Table 4: Hydroxyl scavenging activity of *Salvinia molesta* tertiary form extracts

Sample	Concentration (µg/ml)	% Inhibition	IC_{50} (µg/ml)
Hexane extracts	200	20.52 ± 0.001	830
	400	34.62 ± 0.002	
	600	40.14 ± 0.001	
	800	46.04 ± 0.002	
	1000	53.56 ± 0.004	
Ethyl acetate extracts	200	20.59 ± 0.001	925
	400	32.46 ± 0.002	
	600	34.97 ± 0.002	
	800	38.43 ± 0.001	
	1000	54.69 ± 0.002	
Ethanol extracts	200	14.40 ± 0.008	825
	400	23.69 ± 0.002	
	600	26.65 ± 0.002	
	800	49.54 ± 0.003	
	1000	61.35 ± 0.002	
Methanol extracts	200	29.35 ± 0.003	560
	400	46.32 ± 0.003	
	600	52.32 ± 0.005	
	800	66.25 ± 0.003	
	1000	83.60 ± 0.003	
Ascorbic acid	200	47.07 ± 0.002	400
	400	50.16 ± 0.002	
	600	52.20 ± 0.006	
	800	55.93 ± 0.002	
	1000	62.58 ± 0.001	

Each value represents the mean ± SD of triplicate experiments.

Table 5: H_2O_2 scavenging activity of *Salvinia molesta* secondary form extracts

Sample	Concentration (µg/ml)	% Inhibition	IC_{50} (µg/ml)
Hexane extracts	200	37.80 ± 0.002	380
	400	51.22 ± 0.002	
	600	66.46 ± 0.002	
	800	73.17 ± 0.002	
	1000	80.49 ± 0.002	
Ethyl acetate extracts	200	8.54 ± 0.001	805
	400	22.71 ± 0.002	
	600	38.54 ± 0.002	
	800	53.54 ± 0.001	

	1000	70.63 ± 0.001	
Ethanol extracts	200	43.09 ± 0.001	340
	400	56.10 ± 0.000	
	600	62.60 ± 0.001	
	800	75.61 ± 0.000	
	1000	87.80 ± 0.000	
Methanol extracts	200	14.63 ± 0.002	620
	400	29.27 ± 0.001	
	600	48.17 ± 0.001	
	800	63.41 ± 0.000	
	1000	78.05 ± 0.000	
Ascorbic acid	200	23.33 ± 0.001	445
	400	46.67 ± 0.001	
	600	60.00 ± 0.000	
	800	73.33 ± 0.001	
	1000	83.33 ± 0.001	

Each value represents the mean ± SD of triplicate experiments.

Table 6: H_2O_2 scavenging activity of *Salvinia molesta* tertiary form extracts

Sample	**Concentration (µg/ml)**	**% Inhibition**	**IC_{50} (µg/ml)**
Hexane extracts	200	54.17 ± 0.001	125
	400	62.50 ± 0.001	
	600	73.33 ± 0.001	
	800	78.61 ± 0.001	
	1000	85.56 ± 0.001	
Ethyl acetate extracts	200	7.22 ± 0.001	640
	400	21.67 ± 0.001	
	600	41.39 ± 0.001	
	800	60.00 ± 0.002	
	1000	79.17 ± 0.001	
Ethanol extracts	200	48.43 ± 0.001	320
	400	53.33 ± 0.002	
	600	65.56 ± 0.001	
	800	69.44 ± 0.001	
	1000	73.33 ± 0.000	
Methanol extracts	200	35.83 ± 0.001	520
	400	43.89 ± 0.001	
	600	56.67 ± 0.000	
	800	72.50 ± 0.000	
	1000	85.28 ± 0.001	
Ascorbic acid	200	23.33 ± 0.001	445
	400	46.67 ± 0.001	
	600	60.00 ± 0.000	
	800	73.33 ± 0.001	
	1000	83.33 ± 0.001	

Each value represents the mean ± SD of triplicate experiments.

Table 7: Superoxide anion scavenging activity of *Salvinia molesta* secondary form extracts

Sample	**Concentration (µg/ml)**	**% Inhibition**	**IC_{50} (µg/ml)**
Hexane extracts	200	53.25 ± 0.00	120
	400	59.59 ± 0.00	
	600	60.72 ± 0.00	
	800	62.09 ± 0.03	
	1000	65.90 ± 0.01	

Ethyl acetate extracts	200	22.01 ± 0.00	970
	400	28.93 ± 0.01	
	600	31.98 ± 0.01	
	800	44.80 ± 0.00	
	1000	52.78 ± 0.01	
Ethanol extracts	200	43.27 ± 0.01	570
	400	45.27 ± 0.01	
	600	51.47 ± 0.01	
	800	57.75 ± 0.02	
	1000	59.51 ± 0.00	
Methanol extracts	200	35.58 ± 0.03	420
	400	48.08 ± 0.01	
	600	68.64 ± 0.01	
	800	69.80 ± 0.00	
	1000	71.09 ± 0.00	
Ascorbic acid	200	26.71 ± 0.02	480
	400	31.86 ± 0.08	
	600	58.78 ± 0.04	
	800	75.76 ± 0.04	
	1000	84.56 ± 0.01	

Each value represents the mean ± SD of triplicate experiments.

Table 8: Superoxide anion scavenging activity of *Salvinia molesta* tertiary form extracts

Sample	**Concentration (µg/ml)**	**% Inhibition**	**IC_{50} (µg/ml)**
Hexane extracts	200	24.26 ± 0.00	760
	400	29.20 ± 0.11	
	600	34.66 ± 0.10	
	800	53.89 ± 0.01	
	1000	72.01 ± 0.01	
Ethyl acetate extracts	200	32.07 ± 0.01	920
	400	35.38 ± 0.00	
	600	38.08 ± 0.01	
	800	47.77 ± 0.01	
	1000	52.16 ± 0.00	
Ethanol extracts	200	41.53 ± 0.00	460
	400	47.93 ± 0.01	
	600	51.19 ± 0.00	
	800	63.76 ± 0.02	
	1000	70.93 ± 0.01	
Methanol extracts	200	31.28 ± 0.01	775
	400	36.80 ± 0.03	
	600	47.91 ± 0.01	
	800	53.07 ± 0.04	
	1000	58.16 ± 0.00	
Ascorbic acid	200	26.71 ± 0.02	480
	400	31.86 ± 0.08	
	600	58.78 ± 0.04	
	800	75.76 ± 0.04	
	1000	84.56 ± 0.01	

Each value represents the mean ± SD of triplicate experiments.

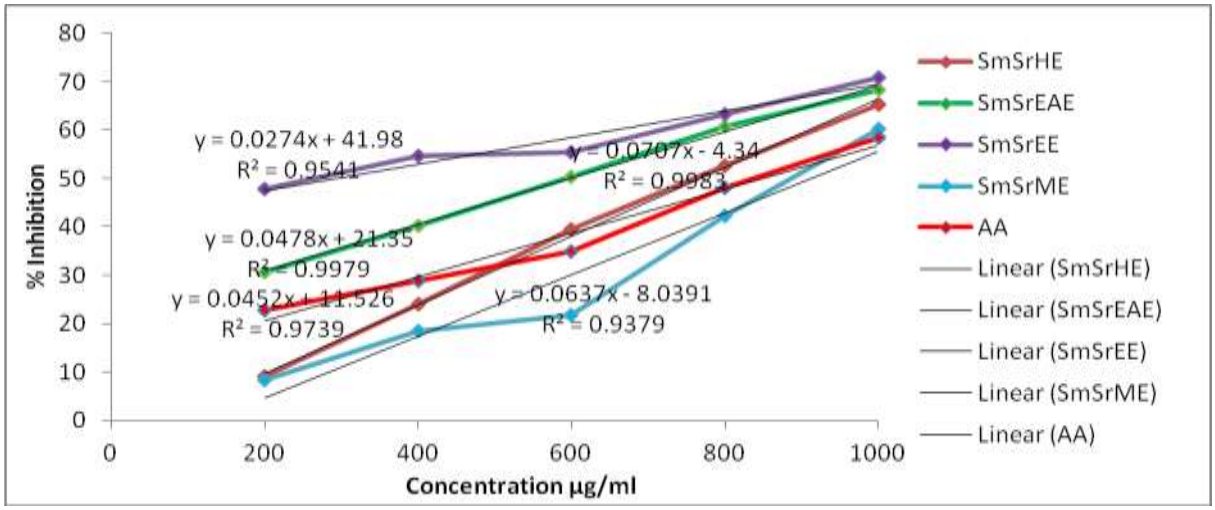

Figure 1: DPPH scavenging activity of *Salvinia molesta* Secondary (SmSr) form extracts.

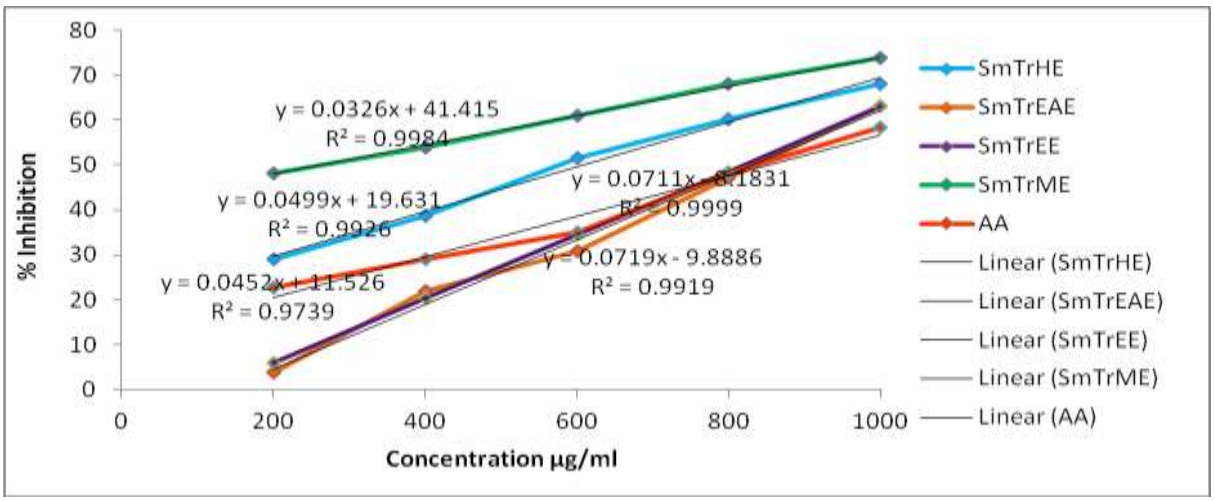

Figure 2: DPPH scavenging activity of *Salvinia molesta* Tertiary (SmTr) form extracts.

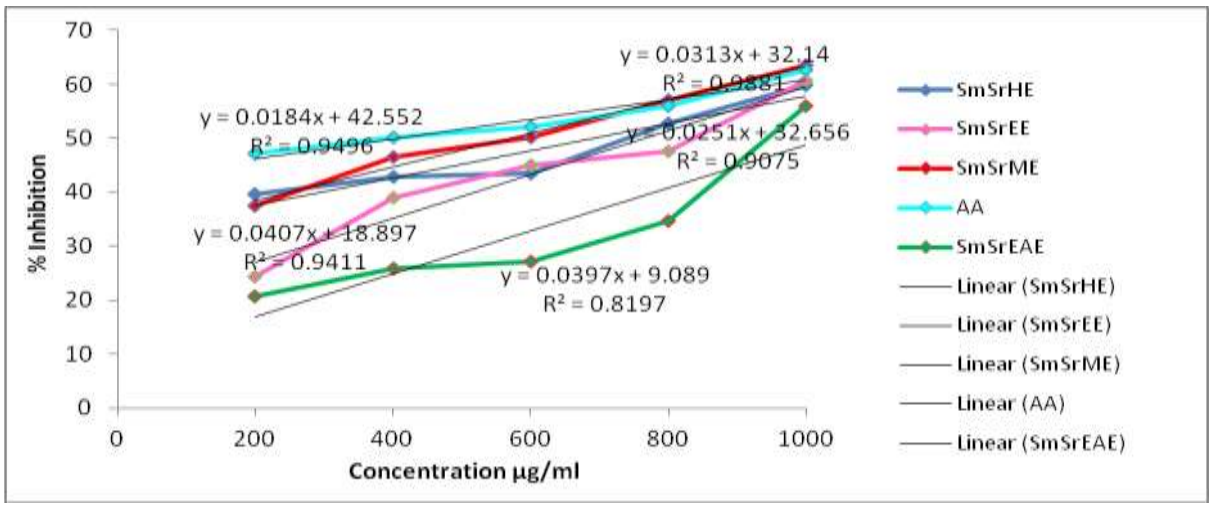

Figure 3: Hydroxyl scavenging effect of *Salvinia molesta* Secondary (SmSr) form extracts.

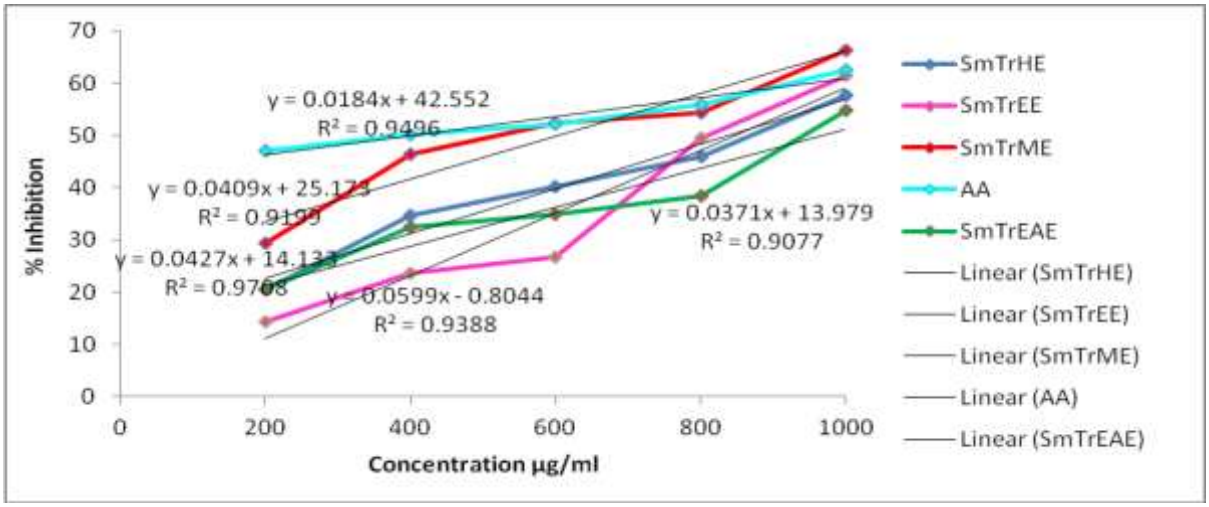

Figure 4: Hydroxyl scavenging effect of *Salvinia molesta* Tertiary (SmTr) form extracts.

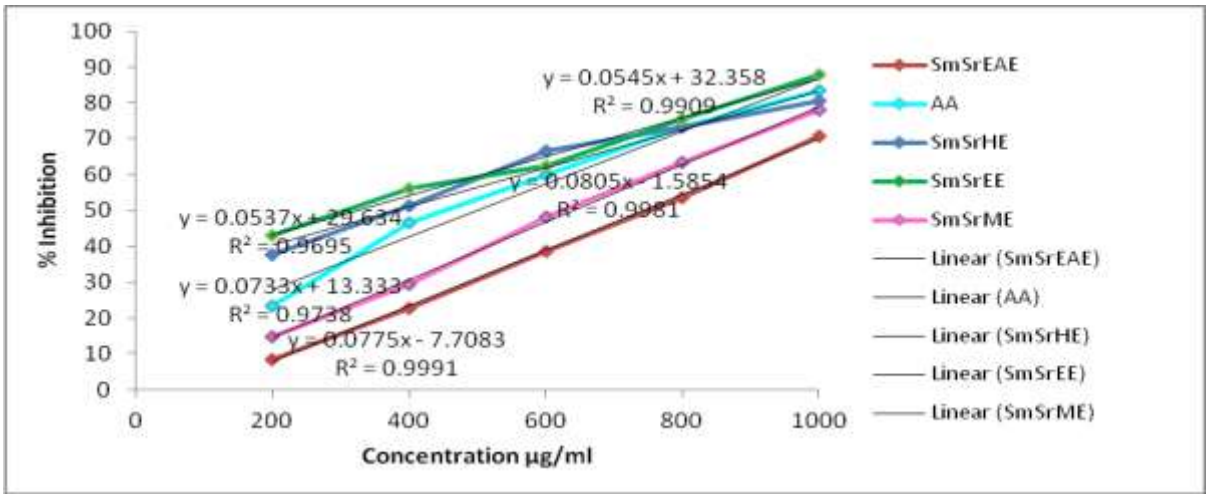

Figure 5: Hydrogen peroxide scavenging potential of *Salvinia molesta* Secondary (SmSr) form extracts.

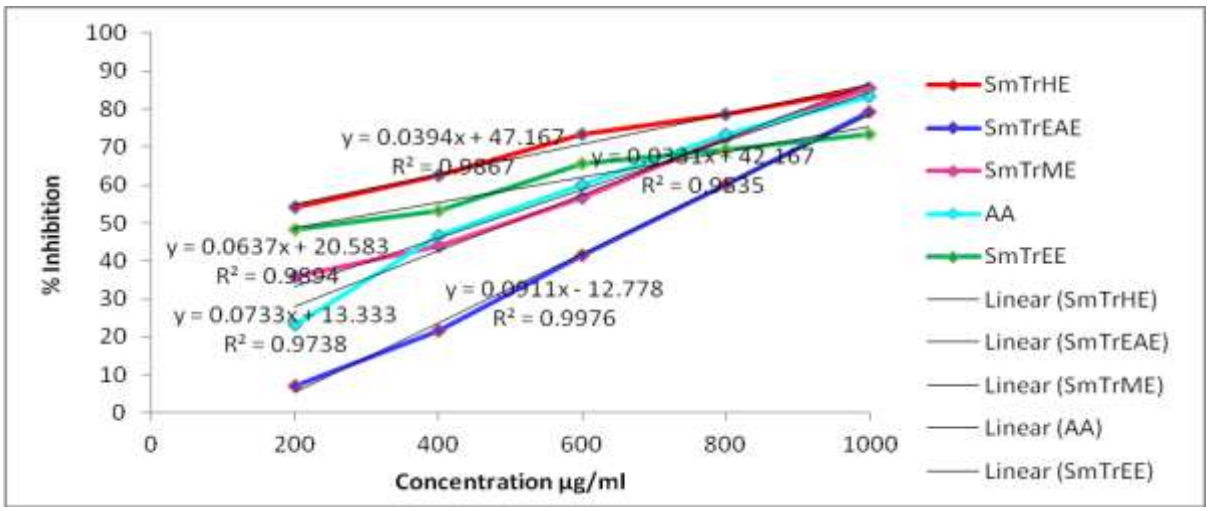

Figure 6: Hydrogen peroxide scavenging potential of *Salvinia molesta* Tertiary (SmTr) form extracts.

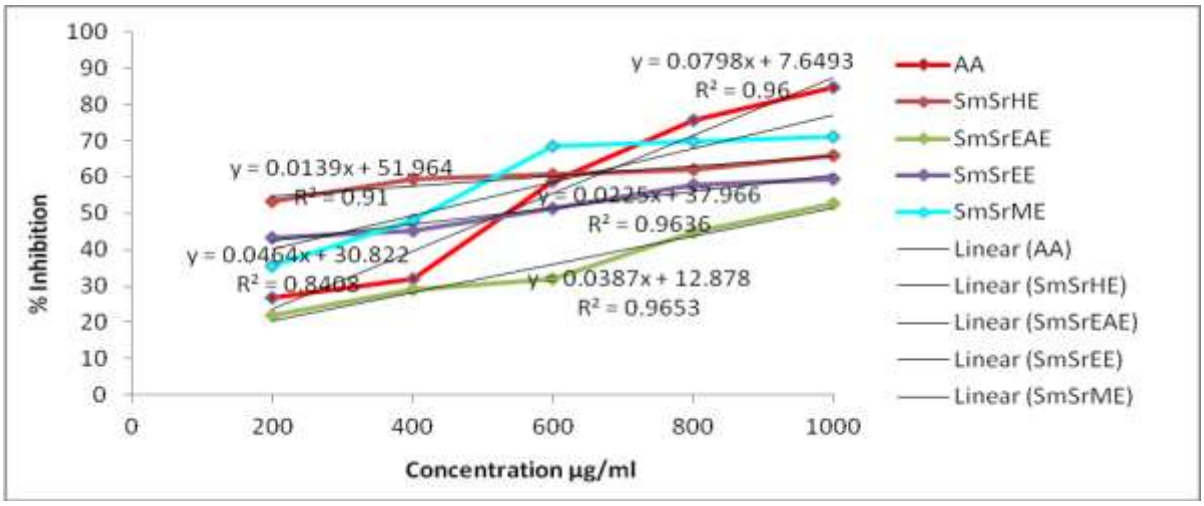

Figure 7: Superoxide anion scavenging effect of *Salvinia molesta* Secondary (SmSr) form extracts.

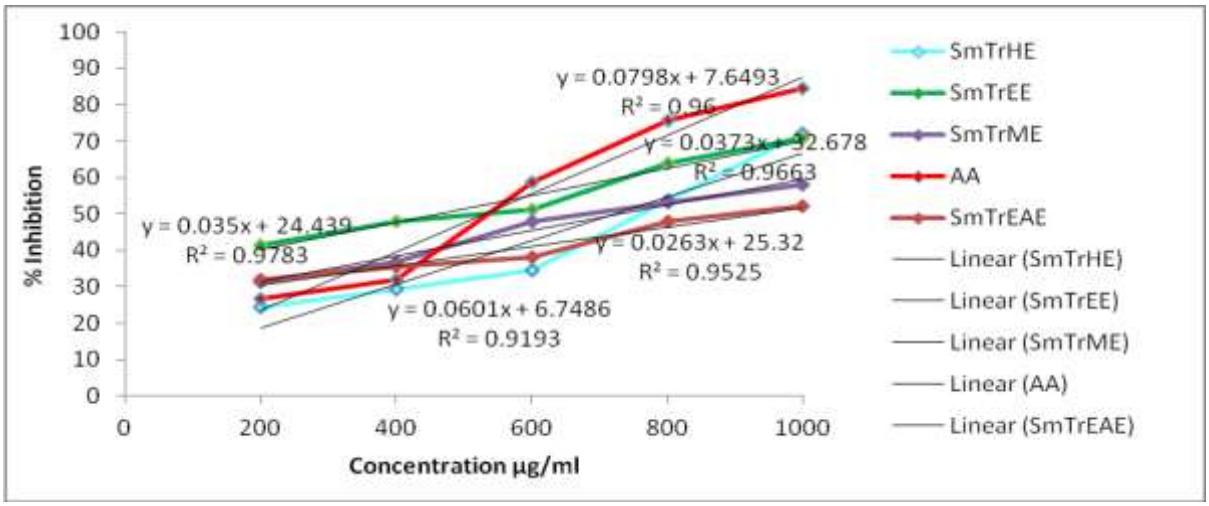

Figure 8: Superoxide anion scavenging effect of *Salvinia molesta* Tertiary (SmTr) form extracts.

REFERENCES

1. Heinrich M, Bames J, Gibbons S. Fundamentals of pharmacognosy and Phytotheraphy. Elsevier Science Ltd., UK 2009.
2. Arnous A, Makris DP, Kefalas P. Effect of principal polyphenolic components in relation to antioxidant characteristics of aged red wines. J. Agric. Food Chem 2001; 49: 5736-5742.
3. Lu F, Foo LY. Toxicological aspects of food antioxidants. In: Madhavi DL, Deshpande SS, Salunkhe DK, Eds; Food antioxidants, Marcel Dekker, New York, 1995; pp. 73-146.
4. Larson RA. The antioxidants of higher plants. Phytochemistry 1998; 27 (4): 969-978.
5. Shahidi F, Naczk M. Food Phenolics: Sources, Chemistry, Effects and Applications. Technomic Pub. Co., Basel, Switzerland, 1995; pp. 331.
6. Cotelle N, Bernier JL, Catteau JP, Pommery J, Wallet JC, Gaydou EM. Antoixidant properties of hydroxyflavones. Free radical Biology and Medicine 1996; 20 (1): 35-43.
7. Velioglu YS, Mazza G, Gao L, Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. Journal of Agricultural and Food Chemistry 1998; 46 (10): 4113- 4117.
8. Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. J. Agri. Food. Chem 2001a; 49: 5165-5170.
9. Cai YZ, Sun M, Corke H. Antioxidant activity of betalains from plants of the Amaranthaceae. Journal of Agricultural and Food Chemistry 2003; 51 (8): 2288-2294.
10. Khan M, Shobha CJ, Rao UM, Sundaram CM, Singh S, Mohan JI, Kuppusamy P, Kutala KV. Protective effect of *Spirulina* against doxorubicin induced cardiotoxicity. Phytother. Res 2005a; 19:1030-1037.
11. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health, International Journal of Biomedical Science 2008; 4: 89-96.

12. Devasagayam TPA, Tilak JC, Boloor KK, Sane KS, Ghaskadbi SS, Lele RD. Free Radicals and Antioxidants in Human Health: Current Status and Future Prospects, JAPI 2004; 52: 795-804.
13. Halliwell B. Free radicals, antioxidants and human disease: curiosity, cause or consequence? Lancet 1994a; 344 (8924): 721-724.
14. Niki E. Free radicals, antioxidants and cancer. In: Ohigashi H, Osawa T, Terao J, Watanabe S, Yoshikawa T, Eds; Food Factors for Cancer Prevention, Springer, Tokyo, 1997; pp. 55-57.
15. Poulson HE, Prieme H, Loft S. Role of oxidative DNA damage in cancer initiation and promotion. European Journal of Cancer Prevention 1998; 7 (1): 9-16.
16. Khan M, Shobha CJ, Rao UM, Sundaram CM, Singh S, Mohan JI, Kuppusamy P, Kutala KV. Protective effect of *Spirulina* against doxorubicin induced cardiotoxicity. Phytother. Res 2005b; 19:1030-1037.
17. Halliwell B. Free radicals, antioxidants and human disease: curiosity, cause or consequence? Lancet 1994b; 344 (8924): 721-724.
18. Mitscher LA, Telikepalli H, McGhee E, Shankel DM. Natural antimutagenic agents. Mutation Research 1996; 350 (1): 142-143.
19. Owen RW, Giacosa A, Hull WE, Haubner R, Spiegelhalder B, Bartsch H. The antioxidant/anticancer potential of phenolic compounds isolated from olive oil. European Journal of Cancer 2000; 36 (10): 1235-1247.
20. Sala A, Recio MD, Giner RM, Manez S, Tournier H, Schinella G, Rios JL. Anti-inflammatory and antioxidant properties of *Helichrysum italicum*. Journal of Pharmacy and Pharmacology 2002; 54 (3): 365-371.
21. Hertog MGL, Kromhout D, Aravanis C, Blackburn H, Buzina R, Fidanza F, Giampaoli S, Jansen A, Menotti A, Nedeljkovic S, Pekkarinen M, Simic BS, Thoshima H, Feskens EJM, Hollman PCH, Katan MB. Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. Archives of Internal Medicine1995; 155 (11): 281-286.
22. Kuo SM. Dietary flavonoid and cancer prevention: evidence and potential mechanism. Critical Reviews in Oncogenesis 1997; 8 (1): 47-69.
23. Mclarty JW. Antioxidants and cancer: the epidemiologic evidence. In: Garewal HS, Eds; Antioxidants and Disease Prevention, CRC Press, New York, 1997; pp. 45-66.
24. Yang CS, Landau JM, Huang MT, Newmark HL. Inhibition of carcinogenesis by dietary polyphenolic compounds. Annual Review Nutrition 2001; 21: 381- 406.
25. Sun J, Chu YF, Wu XZ, Liu RH. Antioxidant and antiproliferative activities of common fruits. Journal of Agricultural and Food Chemistry 2002; 50 (25): 7449-7454.
26. Mitchell DS, Tur NM. The rate of growth of *Salvinia molesta* (*S. aunculata auct*.) in laborator.y and natural conditions. J. Appl. Ecol 1975; 12: 213-225.
27. Mitchell DS, Thomas PA. Ecology of Water Weeds in the Neotropics. Contrib. Intern. Hydrol. Decade, UNESCO, Paris, France, 1972; pp. 50.
28. *Salvinia molesta* – Weed of National Significance [Online resources]. Retrieved from http://www.dpi.nsw.gov.au/agriculture/pests weeds/weeds/profiles/*Salvinia*.[Accessed 22.02.2015].
29. Mitchell DS. The incidence and management of *Salvinia molesta* in Papua New Guinea. Draft Rep., Office Envir. Conserv., Papua New Guinea 1979.
30. Holm LG, Plucknett DL, Pancho JV, Herberger JP. The World's Worst Weeds. Univ. Press of Hawaii, Honolulu, 1977; pp. 609.
31. Chan EWC, Lim YY, Omar M. Antioxidant and antibacterial activity of leaves of *Etlingera species* (Zingiberaceae) in Peninsular Malaysia. Food Chemistry 2007; 104 (4): 1586-1593.
32. Sanchez-Moreno C, Larrauri J, Saura-Calixto F. Free radical scavenging capacity and inhibition of lipid oxidation of wines, grape juices and related polyphenolic constituents. Food Research International 1999; 32: 407-412.
33. Halliwell B, Gutteridge JM, Aruoma OI. The deoxyribose method: a simple "test-tube" assay for determination of rate constant for reactions of hydroxyl radicals. Anal. Biochem 1987; 165: 215- 219.
34. Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Fazelian M, Eslami B. In vitro antioxidant and free radical scavenging activity of *Diospyros lotus* and *Pyrus boissieriana* growing in Iran. Pharmacognosy Magazine 2009; 4(18): 123-127.
35. Robak J, Gryglewski RJ. Flavanoids are scavengers of superoxide anions. Biochem Pharmacol 1998; 37 (5): 837-841.
36. Beauchamp C, Fridovich I. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Anal Biochem1971; 44 (1): 276-287.
37. Umamaheswari M, Chatterjee T. *In vitro* antioxidant activities of the fractions of *Coccinia grandis* L. leaf extract. Afr. J. Trad. Comp. Alter. Med 2008; 5: 61-73.
38. Eklund PC, Langvik OK, Warna JP, Salmi TO, Willfor SM, Sjoholm RE. Chemical studies on antioxidant mechanisms and free radical scavenging properties of lignans. Org. Biomol. Chem 2005; 3: 3336-3347.
39. Ozcelik B, Lee JH, Min DB. Effects of light, oxygen, and pH on the absorbance of 2, 2-diphenyl-1- picrylhydrazyl. J. Food Sci 2003; 68: 487-490.
40. Maisuthisakul P, Suttajit M, Pongsawatmani R. Assessment of phenolic content and free radical scavenging capacity of some Thai indigenous plants. Food Chem 2007; 100: 1409-1418.
41. Hagerman AE, Riedl KM, Jones GA, Sovik KN, Ritchard NT, Hartzfeld PW. High molecular weight plant polyphenolics (tannins) as biological antioxidants. J. Agricul. Food Chem 1998; 46: 1887- 1892.
42. Deivam S, Anbu J, Ravichandran V, Senthil Kumar KL. *In vitro* free radical scavenging potential of poly herbal extract. International Journal of Phytopharmacology 2014a; 5(2): 71-75.
43. Deivam S, Anbu J, Ravichandran V, Senthil Kumar KL. *In vitro* free radical scavenging potential of poly herbal extract. International Journal of Phytopharmacology2014b; 5(2): 71-75.
44. Alves CQ, David JM, David JP, Baha MV, Agular RM. Methods for determination of *In vitro* antioxidant activity for extracts and organic compounds. Qurrnico Nova 2010; 33: 2202-2210.
45. Meyer AS, Isaksen A. Application of enzymes as food antioxidants. Trends Food Sci. Tech 1995; 6: 300- 304.
46. Shirwaikar A, Punitha ISR. Antioxidant studies on the methanol stem extract of *Coscinium fenestratum*. Natural Product Sciences 2007; 13(1): 40-45.
47. Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. J. Agri. Food. Chem 2001b; 49: 5165-5170.