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Anti radical activity and cytoprotective effect of *Sauropaus Androgynous* against oxidative damage induced by CCl_4 in yeast cells

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ABSTRACT

Aqueous extracts of leaves of Sauropaus Androgynous was examined with different in vitro test including diphenly picryl hydrazyl radical scavenging, lipid peroxidation, superoxide anion radical scavenging and reducing power. Aqueous extract showed IC $_{50}$ 1.5 mg/ml on DPPH radical scavenging and 0.4mg/ml to lipid peroxidation of microsomes isolated from rodent liver homogenate induced by the $Fecl_2$- H_2O_2. Superoxide anion and reducing power showed IC $_{50}$ of 0.5 mg/ml, 1.2 mg/ml respectively. Further aqueous extract showed cytoprotective effect against xenobiotic-induced (CCl_4) oxidative stress in yeast cells. The level of Reactive oxygen species, Lipid peroxidation (TBARS) and Lactate dehydrogenase (LDH) generated was significantly lowered in cells treated with extracts along with xenobiotic compared to that of xenobiotic induced cells alone

Keywords: Antiradical activity, cytoprotection, oxidative stress, yeast cells.

INTRODUCTION

Free radical is a molecule that has one or more unpaired electrons. They are formed as intermediates in a normal aerobic life, yet when produced in abundance can harm macromolecules proteins, DNA, carbohydrates.

Oxidative stress, prompted by free radicals, is known to cause a few degenerative maladies, such as, cardiovascular, cataracts, Parkinson, diabetics [1]. Radicals got from oxygen are know as Reactive oxygen species (ROS) successive lessening of atomic oxygen prompts the arrangement of superoxide anion ($.O_2\_$), hydroxyl radical (.OH) and hydrogen peroxide (H_2O_2) which are exceedingly sensitive that targets peroxidation of unsaturated fat present in layer phospholipid prompting harm to membrane lipids, bringing about the gathering of lipid peroxides, which further react with unsaturated fats and proteins [2-3].

Antioxidants are particles that protect cells from the harm brought about by the precarious free radical. They have impact in neutralizing the radical by scavenging ROS, and chelating detoxifying compounds [4]. At present there is a wide interest towards photochemical derived from plants, since they have wide range of wellbeing advancing properties in reducing free radical affected degenerative diseases [5]. Plant derived concentrates involves numerous components including phenolics, flavanoids, tannins, saponins, nitrogen mixes, lignin, glycosides [6]. Natural products containing antioxidants from plants are believed to modulate oxidative stress and to prevent or delay

degenerative disorders [7]. The antioxidant properties of a few plants materials have been reported [8-9]. Therefore there is a thrust for new phytochemicals, antioxidant components that regulate the free radical damage.

Sauropus androgynus belongs to the family Phyllanthaceae is commonly called as multivitamin plant, chakkerumuni, star gooseberry, tropical asparagus, and sweet leaf bush. . In Chinese it is called mani cai, in Malaysia it is called cekur manis or sayur manis, and in Vietnamese, it is called cloth ngot In Karnataka it is called as Chakramuni soppu and is used in making many delicacies and leaves are eaten raw as it has a nutty taste [10]. Leaves of the plants are rich in vitamin A, B and E, proteins and minerals. Sauropus androgynus is generally utilized in preparing nutraceutical items. It is also known to have antibacterial activity [11].

Cytoprotection against natural antioxidants mediated by toxic chemicals, has been reported [12]. In vitro systems using cell culture are useful for studying cytotoxicity mediated by free radicals and to test the cytoprotective action of antioxidants. Cytotoxic injury is believed to be integral to toxicological manifestation and cellular pathobiology. Compounds that ameliorate cytotoxic injury, therefore, are likely to exhibit health-promoting potential [13]. In this study, we have demonstrated the crude extracts of aqueous *Sauropus androgynus* in various *in vitro* antioxidant assays systems, such as DPPH/superoxide scavenging, reducing power and inhibition of lipid peroxidation to understand the amelioration of oxidative stress induced by free radical and cytoprotective action of the extract on yeast cells.

MATERIALS AND METHODS

Extraction

The leaves of *Sauropus androgynus* collected from plant, were washed altogether two to three times with running tap water and after that dried at room temperature. Further, it was cut into little pieces and permitted to dry in dry air at temperature 50^0c for three days. Dried material was grounded to fine powder. Fine powdered material was extracted with luke warm distil water, by keeping in temperature controlled orbital shaker overnight. Later it was filtered utilizing a muslin cloth and further, separated through Whatman No 1 paper, the resultant concentrate was lyophilized to dryness. The concentrates were kept at 4^0C till utilization

Inhibition of Lipid Peroxidation

Liver extracted from grown-up male Wistar rats was homogenized (15-25 g) in 0.02 mol/l tris buffer (pH 7.4). Microsomes were secluded by the strategy depicted by Kamat and Rubin [14]. To 100 µl of liver microsomal suspension, 1 mmol/l each of $Feso_4$ and ascorbic acid were included, with or without extracts in an aggregate volume of 1 ml in 0.1 mol/l phosphate buffer (pH 7.4) and incubated at 37 °c for1 h. Reaction mixture was included with 2 ml each of 20% TCA and 1% TBA, boiled for 10 min, cooled and centrifuged. Malondialdehyde (MDA), which is the side effect of the reaction mixture, was measured at 535 nm.

DPPH radical scavenging assay

The DPPH test was completed as depicted by Guohua et al [15] with some modifications. Aqueous extracts with different concentration were mixed with of 1 ml DPPH solution (0.1 mmol/l, in 95% ethanol (v/v)), and the reaction mixture incubated for 30 min at room temperature. The optical density was measured spectrophotometrically at 517 nm against a blank. Depleting in the absorbance of DPPH indicates a higher radical scavenging activity.

Superoxide radical scavenging assay

Superoxide anion was produced by the response of NADH and phenazine methosulphate (PMS) coupled with a diminishment of Nitro Blue Tetrazolium chloride (NBT) [16]. The reaction mixture contained NBT (0.lmM), NADH (l mM) with or without extracts in an total volume of 1 ml Tris buffer (0.02 M, pH 8.3). The reaction was measured spectrophotometrically at 560 nm each 30 sec for 1 min by including PMS (0.1mM) to the mixture.

Reducing power

The reducing power of the extracts was measured as indicated by the strategy depicted by Oyaizu [17]. 1 ml of reaction mixture containing aqueous extracts Sauropaus Androgynous in phosphate buffer (0.2 mol/l, ph 6.6) was incubated with 3 ml of 1% potassium ferricyanide at 50 °c for 20 min. After incubation, the reaction was ceased by adding 1 ml of 10% TCA solution and the mixture was centrifuged at 3,000 rpm for 10 min. The supernatant was mixed with distilled water (2.5 ml) and ferric chloride solution (0.1 g/ 100 ml), and the absorbance was measured at 700 nm in a spectrophotometer. Higher absorbance indicated higher activity.

Phenol content
Phenolics in the extracts were determined with Folin-Ciocalteu (FC) reagent, as indicated by Yamaguchi [18] utilizing gallic acid as a standard phenolic compound. 1.0 ml of the extract solution containing 1.0 g in a volumetric flask was diluted with distilled water (46 ml), to this 1 ml of FC reagent was included and blended well. After three minutes, 3 ml of 2% Na_2co_3 was added and incubated for 2 h at room temperature. The absorbance was measured at 760 nm. Complete phenolic substance was figured from the standard gallic acid graph.

Cytoprotection
To check the cytoprotection against xenobiotics, carbon tetrachloride (CCl_4) was selected as toxicant, which was used at its lethal concentration at 50 percent (LC_{50}). Cytoprotection experiments were done by incubating 1.0ml of yeast cells (10 X 10^6) suspended in YEPD with xenobiotics (dissolved in DMSO) at LC_{50} concentration 100µM with/without the antioxidant compound for 60 min in a shaking water bath at 37^0C. At the end of incubation, an aliquot of cells was taken for viability assay by the trypan blue exclusion method [19].

Lactate dehydrogenase leakage
After incubation of cells in the presence of xenobiotics with/without the antioxidant compound, cells were centrifuged and the supernatant was assayed for LDH with sodium lactate as the substrate [20].

Lipid peroxidation of yeast cell
After incubation, as above, the cells were centrifuged and the cell pellet was washed in saline and the pellet was boiled in TCA (5.5%) and TBA (0.34%) for 15 min, cooled and centrifuged. The supernatant was measured in a spectrophotometer at wavelength of 535 nm [21].

Reactive oxygen species (superoxide anion)
The cells (10 X 10^6) suspended in 1.0 ml YEPD were incubated with NBT (0.2 mM) with or without xenobiotics (in DMSO) and antioxidant compound in a shaking water bath at 37^0C. The generation of ROS by cells (respiratory burst) was measured by the formation of coloured formazan due to reduction of NBT [22].

Statistical analysis: Data are expressed as mean ± S.E. of three separate experiments.

RESULTS AND DISCUSSION

DPPH
DPPH radical can be measured at absorbance maxima at 517 nm. Fundamentally, it is used to screen the antioxidant activity of different samples. It is a stable free radical, which has ability to donate electrons to looser particles. Decrease in the absorbance indicates the positive effect of the antioxidant activity. The results are shown in Table 1, aqueous extract of *Sauropaus Androgynous* had DDPH radical scavenging activity with an IC_{50} of 1.2mg/ml. however the antioxidant activity was lowered compared to BHA. The free radical scavenging of the extracts is credited to their hydrogen donating capability [18]. Results shows that extract have potential in scavenging the free radical, which could be attributable to its hydrogen giving capacity.

Reactive oxygen species
Inhibitory impacts of aqueous extracts of *Sauropaus Androgynous* on superoxide radicals are shown in Table 1. Inhibition of superoxide radicals with IC_{50} of 0.5 mg/ml was observed in aqueous extracts. BHA was not able to prevent the inhibition of ROS compared to extracts of *Sauropaus Androgynous*. Superoxide radicals are produced amid the ordinary physiological process, for the most part in mitochondria. It is evident that superoxide anion is a feeble oxidant and further experiences oxidation to give hydroxyl radical and singlet oxygen, which are unsafe, prompting oxidative damage [23]. Thus, superoxide radical scavenging by photochemical has physiological ramifications.

Lipid peroxidation
Lipid peroxidation inhibition was seen in aqueous concentrates of *Sauropaus Androgynous* with an IC_{50} of 0.40 mg/ml (Table 1). Oxidation of cell membrane mediated by free radical produces Malondialdehyde (MDA), which is the marker of lipid peroxidation. Cell damage by free radicals, leads to various degenerative diseases viz., atherosclerosis, Parkinson diseases, arthritis [24-25]. Inhibition of lipid peroxidation was observed with the increase in the concentration of the extract, it is evident that the extract may contain certain molecules responsible for

preventing the oxidation of polyunsaturated moiety, by donating the hydrogen molecule by blocking the chain initiation of fatty acid, which is present in cell wall.

Table: 1. Antioxidant activity of the extracts of *Sauropaus Androgynous*

Aqueous extract	Free radical scavenging activity IC_{50} mg/ml				Phenol (mg/g)
	DPPH	ROS	LPO	Reducing activity	
	1.5	0.5	0.4	1.2	12.10±1.01

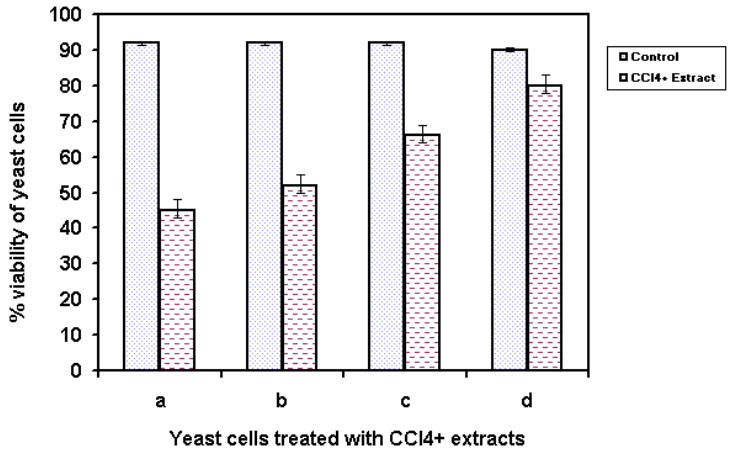

Figure: 1: Cell viability of yeast cell cotreated with different concentration of extracts (a-100µg, b-200µg, c-300µg, d-400µg) and CCl_4 (100 µM)

Reducing power

Aqueous extract showed reducing activity with increasing in the concentration of the extracts, Table 1. Reducing property of the extract is index of antioxidant potential by its ability to donate the hydrogen molecule [26]. Mechanism of total antioxidant activity is involved in various mechanisms viz. binding of transition metal ion, prevention of chain initiation, inhibition of hydrogen abstraction, one such property in reducing activity is associated with the presence of reductones, which play a major role in exerting the antioxidant activity in preventing the formation of hydrogen peroxide by donating the hydrogen atom and preventing the damage caused by free radical. The result obtained suggests that crude extracts has potential biomolecules, which neutralizes the free radicals by donating the hydrogen atom.

Phenolic Content

Phenolic content in the aqueous extract of *Sauropaus Androgynous* was (12.10±1.4 guaicol equivalent per gram). Major role of phenols in scavenging the free radicals is due to the presence of hydroxyl groups. Antioxidant activity of the extract is proportional to the amount of phenol content present in the extract. Several studies on polyphenolic compounds protecting from mutagenesis and carcinogenesis are reported [13].

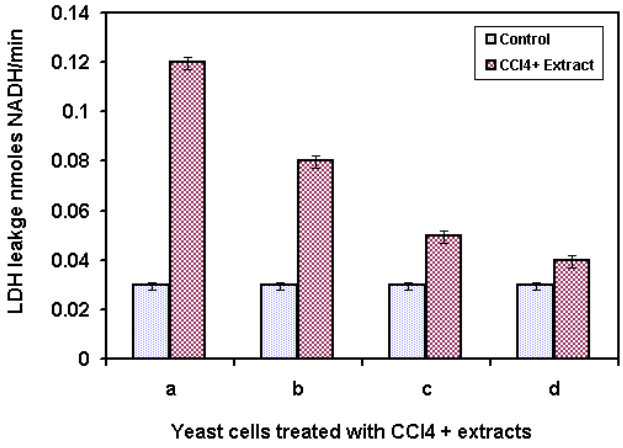

Figure: 2: LDH leakage of yeast cell cotreated with different concentration of extracts (a-100µg, b-200µg, c-300µg, d-400µg) and CCl_4 (100 µM)

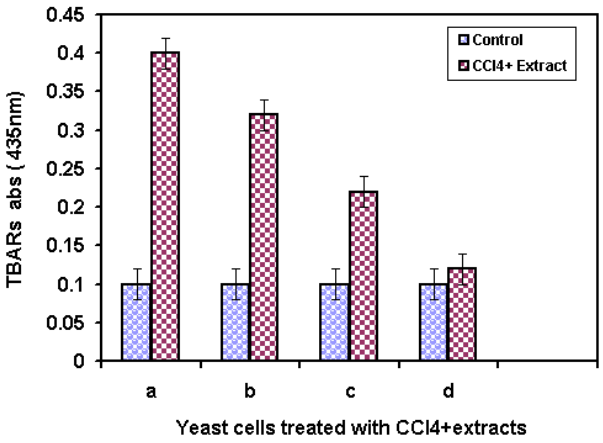

Figure: 3: Lipid peroxidation of yeast cell cotreated with different concentration of extracts (a-100µg, b-200µg, c-300µg, d-400µg) and CCl_4 (100 µM)

Cytoprotective effect of extract on yeast cells against CCl_4 induced damage

In vitro cell cultures play a good model system in understanding the role of the phytochemical in ameliorating the level of oxidative stress induced by xenobiotic in cells, which is measured by viability. Several studies on photochemical have shown cytoprotective effect in both *in vitro* and *in vivo* models [13]. In this experiment we have shown the crude extracts of *S.Andorogynous* in preventing xenobiotic induced cellular damage in yeast cells. CCl_4,

well known inducer of oxidative stress in cells is used as toxicant. Our results showed inhibition of xenobiotic induced lipid peroxidation, inhibition of ROS and preventing cell death with increase in concentration of the extracts The best cell protection, expressed as cell viability, was observed for co treatment with 100–400µg/ml of extract and 100µM CCl_4 Fig.1. LDH secretion in the cells was significantly reduced when cells co treated with increase concentration of extract, compared to CCl_4-treated group Fig.2. Lipid peroxidation was reduced in the cells treated with high concentration of extracts, in which the formation of Malondialdehyde was measured as marker index of lipid bilayer damage Fig 3. Reactive oxygen species (ROS), level increases when cells exposed to stress condition. The level of ROS was depleted when cells cotreated with the extracts Fig 4. These results show that the crude extract may contain cocktail of photochemicals, which ameliorate the level of oxidative stress induced by the CCl_4 and protect the cell undergoing damage. Further study needs to analyze the photochemical responsible for preventing the cell death.

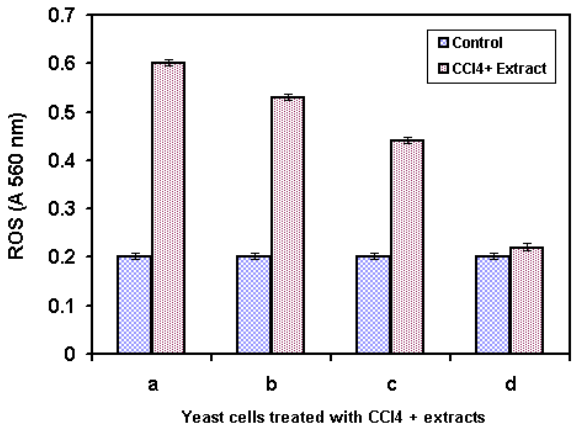

Figure: 4: ROS of yeast cell cotreated with different concentration of extracts (a-100µg, b-200µg, c-300µg, d-400µg) and CCl_4 (100 µM)

CONCLUSION

The present study results suggest that the aqueous extract of *S.Androgynou* may contain various phytochemicals, which can ameliorate different ROS/free radicals under *in vitro* conditions. The wide potential activity of the extracts act as reservoir of nutraceuticals in scavenging free radicals in the counteractive action and improvement of degenerative maladies. Further the extracts have show the cytoprotective action on xenobiotic induced toxicity in yeast cells, which indicates that the extracts has molecules responsible in ameliorating level of oxidative stress caused by the toxicant and preventing the cell death. Though we have not isolated and characterized the antioxidant molecules responsible for antioxidant properties and cytoprotection it could be the phenolic and nonphenolic compound present in the extracts. Further studies in elucidating the antioxidant and cytoprotective molecules need to be analyzed.

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