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Oxidative stress induced radical scavenging, antimicrobial and cytoprotective potential on *Saccharomyces cerevisiae* by ethanolic extract of *Melia dubia* fruit pulp

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Abstract
In this study antioxidant, antimicrobial and cytoprotection activity of crude ethanolic extract of *Melia dubia* fruit pulp was studied. Antioxidant activity was examined in different *in vitro* models, viz., Diphenyl picrylhydrazyl (DPPH) radical, and inhibition of reactive oxygen species (ROS), reducing power, and total phenolic content and lipid peroxidation. Cytoprotective assays performed were cell viability, Reactive oxygen species (ROS), Lipid peroxidation (TBARs), and Lactate dehydrogenase (LDH). Further the extract showed good antimicrobial activity with inhibition in growth up to 80% in different Microorganism, *P. Vulgaris, S. aureus, and E. Aerogens and E. coli*. From the results obtained it was observed that the DPPH radical activity showed IC $_{50}$ of 54 µg, ROS and inhibition of lipid peroxidation with an IC $_{50}$ of 100 and 80 µg respectively. The extract showed good reducing activity, which is an index of hydrogen donor. Also total phenolic content was recorded 71.2 mg/ g of the extract. Further the extract showed cytoprotective action on xenobiotic induced (CCl_4) oxidative stress in *Saccharomyces cerevisiae* (yeast cells), from the obtained results it shows that the ethanolic extract of *M.dubia* is having potent free radical scavenging, antimicrobial and cytoprotection activity suggesting that the ethanolic extracts of *M. dubia* may contain numerous antioxidant molecules, which can effectively scavenge various ROS/free radicals under *in vitro* conditions. The broad range of activity of the extract suggests that *M. dubia* is a source of natural antioxidants, which could be considered as nutraceutical with health promoting properties in the prevention, and amelioration of degenerative diseases

Keywords: *Melia Dubia*, CCl_4, antioxidant, cytoprotection

Introduction
Oxidative stress induced free radicals, damage cells causing several degenerative diseases such as atherosclerosis, diabetes, cancer, aging etc **(**Halliwell & Gutteridge, 1999[1]; Yu, 1994) [2]. Nutraceuticals are good scavengers of free radicals, considered as a good source of food for health benefits. (Klein et al., 2000) [3] Phytochemicals play critical role in reducing the free radical induced degenerative diseases, (Chen & Ho 1995) [4]. There is enormous scope for the plant derived biomolecules, viz., phenolics, flavanodis, lignin, saponins glycosides in neutralizing the radicals and protecting the cells (Kinsella *et al.*, 1993) [5]. Natural products containing antioxidants from plants are believed to modulate oxidative stress and to prevent degenerative diseases. It is believed that higher intake of antioxidant rich food is associated with decreased risk of degenerative diseases (Ames *et al.*, 1993: Joseph *et al*., 1999) [6-7].

In vitro systems using cell culture are useful for studying cytotoxicity mediated by free radicals and to test the cytoprotective action of antioxidants. Cytoprotection against natural antioxidant mediated by toxic chemicals have been reported (Karnick 1993) [8] Cytotoxic injury is believed to be integral to toxicological manifestation and cellular pathobiology (Anup srivastava *et al*., 2007) [9]. Therefore compounds that ameliorate cytotoxic injury are likely to exhibit health-promoting potential.

In the last two decades plants have been considered as a valuable source of natural product towards human health, for various natural therapies (Artizzu *et al.*, 1995) [10]. Plant derived products has been used extensively in pharmaceuticals. As per the World Health Organization, many medicinal plants are source to obtain drugs and cure several ailments. In developed countries about 80% of individuals are using traditional medicines in the form of herbs as they are rich in phenolic, essential oils, tannins (Jansen, *et al.*, 1986, Scalbert, 1991) [11-12] etc. The use of plant extracts or photochemical has significance in antimicrobial properties. There are several reports on plants for their antimicrobial Paction (Saxena *et al*., 1994) [13].

Melia dubia belongs to family *Meliaceae*, mainly distributed in Asian countries (India, Srilank, Malaysia, and Australia). It is a tree which grows tall up to 25m, flowers/fruits between March to April (Gopal *et al*., 2015) [14]. *M.dubia* as several chemical like alkaloids, glycosides, saponins, resins, oleoresins, lactones and essential oils (Valentina *et al*., 2013)[15]. According to literature review *M.dubia* has many pharmacological properties; hepatoprotective, antiulser, anti-inflammatory, antifeedant, analgesic, antidiabetic, anticancer, and biopesticidal activities (Dayanajayaleela *et al*., 2016) [16]. Since the plant is having the potent molecules, study was conducted to evaluate its free radical scavenging potential in different *in vitro* models and antimicrobial action. Further, cytoprotection study was also performed to check that the ethanolic extract of *M.dubia* fruit pulp in protecting the oxidative stress induced damage in yeast cells by ameliorating the level of free radicals

Materials and Methods

Extraction

M.dubai was washed thoroughly with tap water for two to three times and fruit pulp was scraped with knife and was allowed to dry at room temperature. Further the dried pulp was broken into small pieces and grounded into fine powder using grinder/mixer. Powdered material was extracted with ethanol using Soxlet apparatus. The extractant was subjected to flash evaporator to dry the solvent and the extract obtained was stored at 4^0C till use.

Inhibition of Lipid Peroxidation

Microsomes were isolated by the method described by (Kamet and Rubin 1972) [17]. To 100 µl of liver microsomal suspension, 1 mmol/l each of FeSo4 and ascorbic acid were added, with or without extracts in a total volume of 1 ml in 0.1 mol/l phosphate buffer (pH 7.4) and incubated at 37 °C for 1 h. After incubation, the reaction mixture was added with 2 ml each of 20% TCA and 1% TBA, followed by heating in a water bath for 10 min, cooled and centrifuged. Malondialdehyde (MDA), which is the byproduct of the reaction mixture, was measured at 535 nm.

DPPH radical scavenging assay

The DPPH assay was carried out as described by (Guohua et al 1997) [18] with some modifications. Different concentrations of ethanolic extracts 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. BHA was used as a positive control. Decrease in the absorbance of DPPH indicates a higher radical scavenging activity.

Superoxide radical scavenging assay

Superoxide anion was generated by the reaction of NADH and phenazine methosulphate (PMS)

Coupled with a reduction of Nitro Blue Tetrazolium chloride (NBT) (Nishikimi *et al.*, 1972) [19]. The reaction mixture contained NBT (100 lM), NADH (300 lM) with or without extract in a total volume of 1 ml Tris buffer (0.02 M, pH 8.3). The reaction was measured spectrophotometrically at 560 nm every 30 sec for 1 min by adding PMS to the mixture

Reducing power

The reducing power of the extracts was measured according to the method described by (Oyaizu 1986) [20]. 1 ml of reaction mixture containing extracts 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 stopped 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 increased reducing power.

Antimicrobial activity

Antimicrobial activity of ethanolic extract of *M.dubia* fruit pulp extract was performed by Spectrophotometric method (Jansen, *et al.*, 1986) [11]. To the 5ml of nutrient broth different concentration of *M.dubia* pulp extract was added followed by inoculating with of different bacterial strain (*E.coli, E.aerogens. S.aureus, P.vulgaris*) respectively. Inoculated tubes were incubated at 37^0C for 24-48hours in incubator, further the absorbance was measured at 700nm against blank.

Cytoprotection

Cell viability

CCl_4 was used as toxicant, to check the cytoprotection of the extract. The concentration of CCl_4 was used at 50 percent lethality (LC_{50}). Cytoprotection investigations were performed by incubating 1.0 ml of yeast cells (10×10^6) suspended in YEPD with CCl_4, (dissolved in DMSO) at LC50 concentration 1mM with/without the extract for 1 hr in a shaking water shower at 37^0C. After the incubation period, an aliquot of cells was taken for viability test by the trypan blue exclusion method (Anup *et al.*, 2007) [9].

Lactate dehydrogenase leakage

The supernatant obtained after incubation followed by centrifugation from the reaction mixture of yeast cells in the presence of xenobiotics with/without extract was assayed for LDH with sodium lactate as the substrate (Bergmeyer 1974) [21].

Lipid peroxidation of yeast cell

The cells were centrifuged after incubation, with CCl_4 along with the extract 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 (Cereser *et al.*, 2001) [22].

Reactive oxygen species (superoxide anion)

The cells (10×10^6) suspended in 1.0 ml YEPD were incubated with NBT (0.2 mM) with or without xenobiotics (in DMSO) and extracts 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 (Pompeia *et al.*, 2003) [23].

2.7 Statistical analysis

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

Results and Discussion

DPPH Radical Scavenging Activity

DPPH is a free radical molecule which has affinity to accept electron and becomes stable. Basically, it is used to screen the antioxidant activity of various samples. DPPH radical can be

measured at absorbance maxima at 517 nm. Decrease in the absorbance indicates the acceptance of electrons which is induced by the antioxidants. The results are shown in Table. 1 with IC_{50} of 54µg/ml. The antioxidant activity of the extracts is credited to their hydrogen donating ability (Yamaguchi *et al.*, 1998) [24]. Results suggest extracts have strong potential in scavenging the free radical, which could be attributable to its hydrogen donating ability.

Superoxide Radical Scavenging

Inhibitory effects of ethanolic extract of *M. Dubia* fruit pulp on superoxide radicals are shown in Table.1. Scavenging of superoxide radicals with IC_{50} of 100 µg/ml was observed in aqueous extract. Superoxide radicals are generated during the normal physiological process, mainly in mitochondria. It is evident that superoxide anion is a very weak oxidant, undergoes oxidation to give hydroxyl radical and singlet oxygen, which are harmful, leading to oxidative stress (Dahl and Richardson 1978) [25]. Therefore, superoxide radical scavenging by antioxidants has physiological implications.

Table 1: Antioxidant activity of ethanolic extract of *M. dubia fruit pulp*

Ethanolic extract	Free radical Scavenging activity IC_{50} µg/ml			Phenol mg/g
	DPPH	ROS	LPO	71.2±1.4
	54	100	80	

Lipid Peroxidation

Inhibition of lipid peroxidation was observed in extracts of *M.dubia* fruit pulp with an IC_{50} of 80µg/ml (Table.1). Oxidation of polyunsaturated fatty acids in the cell membrane produces Malondialdehyde (MDA), which is the index of lipid peroxidation and marker of cell injury. Cell damage can occur in any internal organ by free radicals, leading to various disorders, viz., atherosclerosis, hepato and nephro damage (Janero, 1990; Rice-Evans and Burdon 1993) [26-27]. From the results it was observed that inhibition of lipid peroxidation is concentration dependent of extracts, indicating ethanolic extract has certain antioxidant molecules which are able to repair the damage caused by the free radicals. The mechanism in inhibiting the lipid peroxidation by the extract could be by preventing the chain initiation of polyunsaturated fatty acid chain by donating the hydrogen atom to the damaged lipid bilayer.

Reducing Power

The reducing power of the extract values are shown in Table.1. The extracts showed increasing activity with increase in the concentration of extracts. Reducing capacity of the extracts could be considered an indicator towards its potential antioxidant properties. Potency of antioxidant may be due various mechanism viz., prevention of chain initiation, binding of transition metal ion, inhibition of hydrogen abstraction, radical scavenging and preventing lipid bilayer damage (Diplock, 1997; Duh 1998) [28,29] has shown that reducing properties are associated with the presence of reductones. Reductones has significant role in scavenging the free radical by donating hydrogen molecule and preventing the cell damage. Results suggest that the *M.dubia* extract have potential to scavenge free radical damage by donating hydrogen atom thereby preventing oxidative stress.

Phenolic Content

Phenolic content in the ethanolic extract of *M. dubia* fruit pulp was (71.2±1.4 mg gallic acid equivalent per gram, respectively). Phenols play a major role in radical scavenging because of their hydroxyl groups. Total phenolic content present in the extract is directly related to antioxidant activity [29]. In our study, there is a correlation between antioxidant activity and phenol content. The various antioxidant activities of ethnaloic extract of *M. dubia* demonstrated in this study clearly indicates the potential application value of *M. dubia*.

Cytoprotection of M. dubia fruit pulp on yeast cells against CCl_4, induced damage

In vitro cell culture play a model role in evaluating the phytochemical in ameliorating the level of oxidative stress induced by xenobiotic in cells, which is measured by cell viability. Earlier studies on photochemical have shown cytoprotection in both *in vitro* and *in vivo* models (Anup *et al*., 2007) [9]. In this test we have demonstrated the *M.dubia* extract in protecting xenobiotic induced cell death in yeast cells. CCl_4, inducer of oxidative stress in cells is utilized as toxicant. Outcomes of result indicated depletion of xenobiotic induced lipid peroxidation, inhibition of ROS by preventing cell death and reduction in level of LDL leakage with increase in concentration of extract. Cytoprotection observed as cell viability, was observed for cells co treatment with 50–200µg/ml of extract and 1mM CCl_4, Fig.1. LDH leakage in the cells was altogether decreased when cells co treated with increasing concentration of extract, compared with CCl_4, treated Fig.2. Lipid peroxidation was depleted in the cells treated with high convergence of extracts, in which the development of Malondialdehyde was measured as marker record of lipid bilayer damage Fig 3. Reactive oxygen species (ROS), level increases when cells exposed to stress condition. The level of ROS was reduced when cells co treated with the extracts Fig 4. These outcomes demonstrate that the unrefined extract might contain cocktail of phytochemicals, which improve the level of oxidative stress instigated by the CCl_4, by protecting the cell from undergoing death. Further the photochemical responsible from protecting cell death, work under progress.

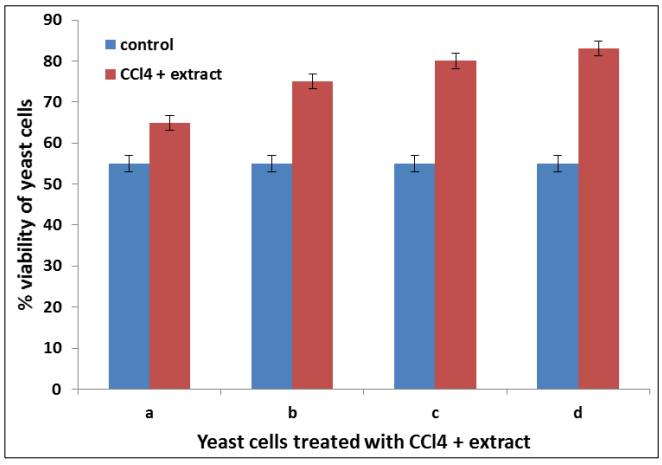

Fig 1: Cell viability of yeast cell co treated with different concentration of extracts (a-50□g, b-100□g, c-150□g, d-200□g) and CCl_4 (1mM)

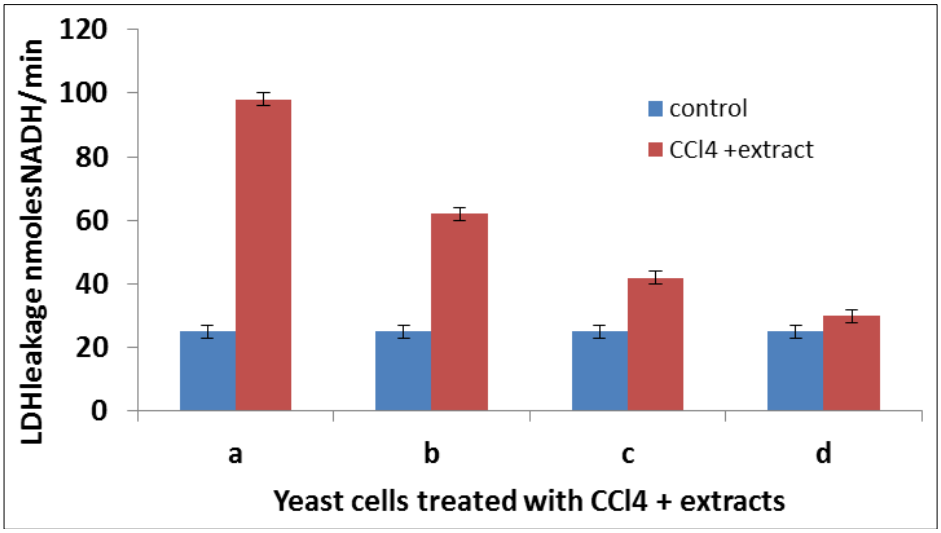

Fig 2: LDH leakage of yeast cell co treated with different concentration of extracts a-50□g, b-100□g, c-150□g, d-200□g) and CCl_4 (1mM)

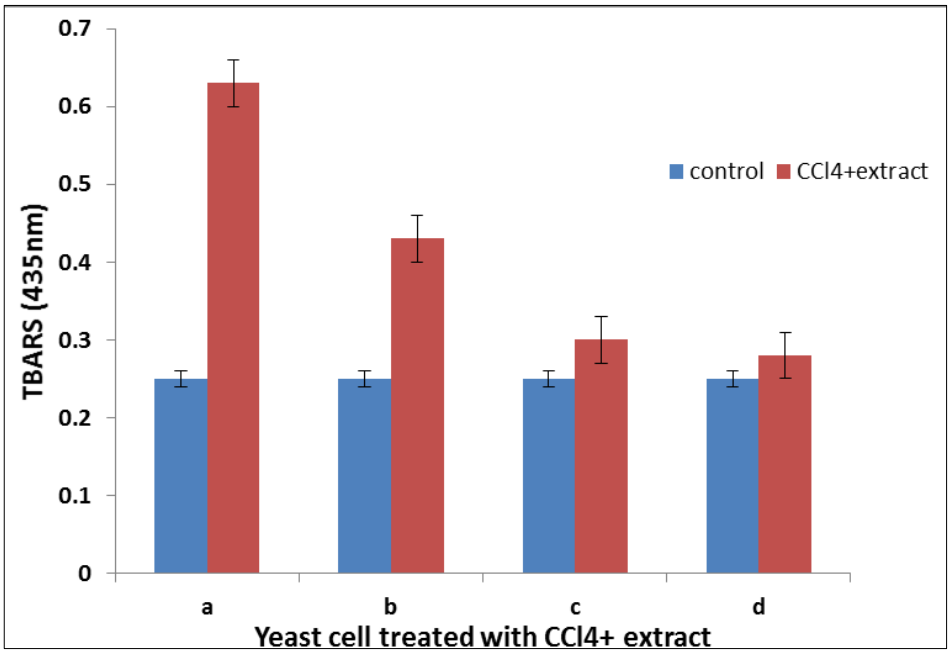

Fig 3: Lipid peroxidation of yeast cell co treated with different concentration of extracts a-50□g, b-100□g, c-150□g, d-200□g) and CCl_4 (1mM)

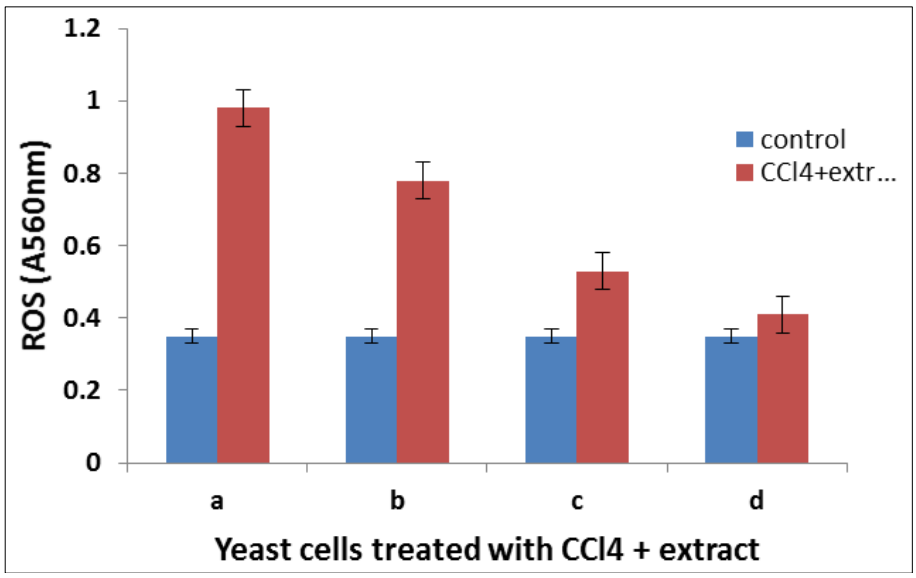

Fig 4: ROS of yeast cell cotreated with different concentration of extracts a-50□g, b-100□g, c-150□g, d-200□g) and CCl_4 (1mM)

Conclusion

The results obtained in the present study clearly suggest that the ethanolic extract of *M.dubia* may contain numerous antioxidant compounds which can effectively scavenge various ROS/free radicals under *in vitro* conditions. Further the extracts have show the cytoprotective action on xenobiotic induced toxicity in yeast cells, caused by the toxicant and preventing the cell death The broad range of activity of the extract suggests that *M.dubia* is a source of natural antioxidants which could be considered as nutraceutical with health promoting properties in the prevention and amelioration of degenerative diseases. Although we have not isolated and characterized the antioxidant molecules responsible for the antioxidant activity and cytoprotection, we speculate that it could be related to the phenolic and nonphenolic compounds present in the extract. Therefore, further work will be carried out to isolate and identify the effective cytoprotective molecules.

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References

1. Halliwell B, Gutteridge, JMC. Role of free radicals and catalytic metal ions in human disease: an overview. Met in Enzymol. 1990; 186:1-85.
2. Yu BP. Cellular defenses against damage from reactive oxygen species. Physiol Rev. 1994; 76:139-162.
3. Klein C, Sato T, Meguid MM, Miyata G. From food to nutritional support to specific nutraceuticals: A journey across time in the treatment of disease. J Gastroenterol. 2000; 35:1-6.
4. Chen CW, Ho CT. Antioxidant properties of polyphenols extracted from green tea and black tea. Journal of Food Lipids. 1995; 2:35-46.
5. Kinsella JE, Frankel E, German, B, Kanner J. Possible mechanisms for the protective role of antioxidants in wine and plant foods. Food Technol. 1993; 4:85-89.
6. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants and the degenerative diseases of aging. Proceedings of the National Academy of Sciences of the United States of America. 1993; 90:7915-7922
7. Joseph JA, Shukitt-Hale B, Denisova NA, Bielinski D, Martin A, McEwen, JJ. Et al. Reversals of agerelated declines in neuronal signal transduction, cognitive, and motor behavioural deficits with blueberry, spinach, or strawberry dietary supplementation. Journal of Neuroscience. 1999; 19:8114-8121.
8. Karnick CR. Pharmacopoeial Standards of Herbal Plants. New Delhi, India: Sri Satguru Publications, 1994.
9. Anup Srivastava, Jagan Mohan Rao L, Shivanandappa T. Isolation of ellagic acid from the aqueous extract of the roots of *Decalepis hamiltonii*: Antioxidant activity and cytoprotective effect. Food Chem, 2007; 103:224-233.
10. Artizzu N, Bonsignore L, Cottiglia F, Loy G. Studies of the diuretic and antimicrobial activity of *Cynodon dactylon* essencial oil. *Fitoterapia*; 1995; 66:174-175.
11. Jansen, AM, Cheffer, JJC, Svendsen, AB. Antimicrobial activity of essencial oils: a 1976-1986 literature review. Aspects of test methods. Planta Med.1987; 40:395-398,
12. Scalbert A. Antimicrobial properties of tanins. Phytochem. 1991; 30:3875-3883.
13. Saxena G, McCutcheon AR, Farmer S, Towers GHN, Hancock REW. Antimicrobial constituents of Rhus glabra. J Ethnopharmacol.1994; 42:95-99.
14. Gopal V, Prakesh Yoganandan G, Manju P. Concise review on *Melia Dubia* (Meloaceae). Eur J Environ Ecol, 2015; 2:57-60.
15. Valentina P, illango KI, Kirutiga B, Parimala MJ. Preliminary phytochemical analysis and Biological screening of extract of leaves of *Melia Dubia* cav. Int J Res Ayurveda pharam. 2013; 4:417-9.
16. Dayanajayaleela G, IrdayaMonisha S, Anitha Immaculate A, Rosaline Vimala J. Studies on hytochemical, nutritional analysis and screening of *in vitro* biological activities of *Melia Dubia* leaf extract. Int J Sci Eng Res. 2016; 7:56-68.
17. Kamath SA, Rubin E. Interaction of Calcium with Microsomes: A Modified Method for the Rapid Isolation of Rat Liver Microsomes, Biochemical and Biophysica Research Communications. 1972; 49(1):52-59.
18. Guohua C, Emin S, Ronald L. Antioxidant and Prooxidant Behavior of Flavonoids: Structure-Activity Relationship", Free Radical Biology and Medicine. 1997; 22:749-760.
19. Nishikimi M, Rao A, Yagi K. The Occurrence of Superoxide Anion in the Reaction of Reduced Phenazine Methosulphateand Molecular Oxygen, Biochemical and Biophysical Research Communications. 1972; 46:849-854.
20. Oyaizu M, Studies on Product of Browning Reaction Prepared from Glucose, Amine. Japanese Journal of Nutritio. 1986; 44:307-315.
21. Bergmeyer HU. Methods of enzymatic analysisWeinheim: Verlag Chemie. 1974; 2:556–760.
22. Cereser C, Boget S, Parvaz P, Revol A. Thiraminduced cytotoxicity is accompanied by a rapid and drastic oxidation of reduced glutathione with consecutive lipid peroxidation and cell death. Toxicol. 2001; 163:153-162.
23. Pompeia C, Cury-Boaventura MF, Curi R. Arachidonic acid triggers an oxidative burst in leukocytes. Braz J Med Biol Res. 2003; 36:1549-1560.
24. Yamaguchi T, Takamura H, Matoba T, Terao J. HPLC Method for Evaluation of the Free Radical-Scavenging Activity of Foods by Using 1, 1-Diphenyl-2-Picrylhydrazyl Bioscience, Biotechnology, and Biochemistry. 1998; 62(6):1201-1204.
25. Dahl MK, Richardson T. Photogeneration of Superoxide Anion in Serum of Bovine Milk and in Model Systems Containing Riboflavin and Amino Acids", Journal of Dairy Science. 1978; 61(4):400-407.
26. Janero D. Malondialdehyde and Thiobarbituric Acid-Reactivity as Diagnostic Indices of Lipid Peroxidation and Peroxidative Tissue Injury, Free Radical Biology and Medicine. 1991; 9(6)515-540.
27. Rice-Evans C, Burdon R. Free Radicals-Lipid Interaction and Their Pathological Consequences", Progress in Lipid Research. 1993; 32, 1:71-110.
28. Diplock AT. Will the 'Good Fairies' Please Proves to us that Vitamin E Lessens Human Degenerative of Disease?" Free Radical Research. 1997; 27(5):511-532.
29. Duh PD. Antioxidant Activity of Burdock (*Arctium lappa* Linne): Its Scavenging Effect on Free Radical and Active Oxygen, Journal of the American Oil Chemist's Society. 1998; 75(4):455-461.