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EFFECT OF *Solanum nigrum* AND *Emblica officinalis* FRUIT EXTRACTS AGAINST H_2O_2 INDUCED OXIDATIVE STRESS IN *Saccharomyces cerevisiae*

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ABSTRACT

In this study, the effect of *Emblica officinalis* and *Solanum nigrum* fruit extracts against H_2O_2 induced oxidative stress in *Saccharomyces cerevisiae* was analyzed using vitamin E as standard. GC-MS analysis of ethanolic extract of *S.nigrum* and *E.officinalis* fruits revealed the presence of 51 and 25 phytoconstituents respectively. *S.cerevisiae* cells cultured in YEPD medium were divided into eight groups of $1X10^6$ cells / ml each. The groups pre-treated with plant extract were subjected to oxidative stress by H_2O_2. Decreased apoptosis was observed with the yeast cells treated with fruit extract as compared with the cells treated with H_2O_2 alone. Antioxidant nature of the fruit extracts was determined by assessing SOD and catalase activity in yeast cells. The level of protein, SOD and catalase activity showed significant increase in yeast cells treated with plant extract than H_2O_2 treated cells. These extracts also protected the cells, its protein and other biomolecules from the oxidative damage, thereby increased the cell viability and stability. The protective mechanism may be due to the phytoconstituents with antioxidant nature and hence these plant fruits could be used as an alternate as therapeutic agents in free radical mediated diseases.

KEYWORDS: ***S.nigrum, E.officinalis, S.cerevisiae*** **and phytoconstituents.**

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INTRODUCTION

Oxidative stress as a result of free radicals can damage molecular targets such as DNA, protein and lipids[1], which appears to be the basic mechanism underlying various disorders such as neurodegenerative disorders, diabetes, inflammation, viral infections and autoimmune disorders. An antioxidant is an agent or molecules which could slowdown or prevent the oxidation of other molecules. Plants with antioxidant properties have long been related to their therapeutic effects in Parkinson's disease, Alzheimer's disease, viral infections and inflammation [2]. Medicinal plants are continue to be the rich sources of natural antioxidants [3], which are exploited to prevent different types of stress-related diseases and aging which are closely associated to free radicals. *E. officinalis*, a highly valued plant in the Indian traditional medicine system[4]. The dried fruits of amla in Unani and Ayurvedic medicine have been reported for the treatment of haemorrhage, diarrhoea and dysentery [5]. *E. officinalis* fruits contain tannin principles such as emblicanin A, emblicanin B, punigluconin and pedunculagin. Ethanolic extract of the different parts of *S. nigrum* found to have hydroxyl radical scavenging ability, this property forms the basis for the mechanism of cytoprotection[6]. The specific objectives of the study was to assess the phytoconstituents and the cytoprotective effects of *E.officinalis* and *S.nigrum* fruit extracts on oxidative stress induced apoptosis in *S. cerevisiae.* Reactive Oxygen Species (ROS) are eliminated by different enzymic and non-enzymic antioxidants in cells. SOD converts the super oxide anion to H_2O_2, which in turn detoxified to water by catalase or glutathione peroxidase. These two enzymes act as best defense system against the free radicals. *S. cerevisiae* has been established as a suitable experimental model for investigating basic molecular mechanisms in eukaryotes, e.g. cell cycle progression[7] and as they possesses the basic machinery of apoptosis. As the yeast is a eukaryotic model, it can be upgraded for leading research into different kinds of human disease. *S. cerevisiae* has been used to study the cellular GSH level, mitochondrial function to evaluate inhibition of cellular growth, cytotoxicity and genotoxicity induced by different drugs[8]. Hence, this study was carried out using *S. cerevisiae* as the experimental model to study the cytoprotective effect of the selected fruit extracts.

MATERIALS AND METHODS

Collection of plant materials

The fruits of *E. officinalis* and *S.nigrum* were collected from Kumbakonam, Thanjavur District, Tamil Nadu, India. The fruits of both the plants were shade dried for five days and they were powdered with electrical blender. The material was stored in an airtight container at room temperature till the use.

Preparation of fruit extracts

100 g of powdered fruit material of each plant was taken in a clean glass container and immersed in 500 ml of petroleum ether. After 48 hrs the content was filtered using Whatman filter paper. To the remaining plant material, 500 ml of ethanol was added and mixed well. The content was kept sealed for 7 days with occasional shaking and stirring. The whole mixture then subjected to coarse filtration by a piece of clean cotton material followed by Whatmann filter paper. The filtrate was evaporated to dryness under vacuum in a rotary shaker and extract obtained was stored at -20° C till further use. The plant extracts prepared by cold maceration method were dissolved in water and DMSO and were used for phytochemical analysis by GC-MS and oxidative studies respectively.

Analysis for phytoconstituesnts

The extracts of *S.nigrum* and *E.officinalis* were analyzed for secondary metabolites qualitatively by the method of Gopish Khanna[9].

GC-MS analysis of ethanolic extracts

The GC – MS analysis was carried out using a Clarus 500 Perkin – Elmer consisting Capillary Column Elite-5MS (5%Phenyl 95% dimethylpolysiloxane) with 30m length and id 250 µm. The instrument was set to an initial temperature of 50°C and raised @ 7ºC/min to 220ºC. Finally, the temperature was raised to 280°C and it was maintained for 10 min. up to rate, which is . The ionization voltage was 70eV. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 45-450 (m/z). GC-MS chromatogram of *S.nigrum* and *E.officinalis* fruits extract indicated the presence of about 51 and 25 phytoconstituents respectively. The GC-MS spectra of these extracts was compared with NIST library. . The phyto constituents identified from *S.nigrum* and *E.officinalis* fruit extracts are represented in Table 2 and 3 respectively.

Studies on Saccharomyces cerevisiae with plant extract

S cerevisiea grown in Yeast Extract Peptone Dextrose (YEPD) medium at 37° C and were divided into 8 groups of 1X 10^6 cells/ml. Control group treated with no plant extract, Group 2 H_2O_2 control, Group 3 and 4 were treated with 25µg/ml and 50 µg/ml of *E.officinalis* extract respectively. Similarly, group 5 and 6 were treated with the above concentrations with *S.nigrum* extract. As a positive control group 7 and 8 were treated with vitamin E at 25µg/ml and 50µg/ml respectively. All the groups were treated, except Group1, with H_2O_2 (200µM) after one hour of plant extract or vitamin E treatment and incubated at 37° C for 24 hrs. The yeast cells recovered after 24 hrs were used for the preparation of cell extract to assess the antioxidant nature of these extracts. A small aliquot of yeast cells from each group recovered for Giemsa staining[10], which was used to differentiate nuclear and cytoplasmic morphology of cells. The apoptotic ratio was calculated using the formula.

$$\text{Apoptotic ratio} = \frac{\text{No. of apoptotic cells}}{\text{No. of normal cells}}$$

Biochemical analysis

The amount of protein[11], activity of catalase[12] and superoxide dismutase (SOD)[13] were determined in yeast cell homogenate.

Statistical analysis

Results were expressed as mean ± SD for six experiments in each group. Statistical analysis was carried out using Student's –t- test. Statistically significant variations are compared as follows: control (Group1) vs H_2 O_2 treated (Group2) and (Group2) vs (Group 3-8). Results were considered significantly different if $P < 0.05$.

RESULTS

Analysis of Phytoconstituents

Qualitative analysis of crude ethanolic extracts of *E.officinalis* and *S. nigrum* fruits was carried out and the results obtained were represented in Table 1. Both the extract revealed the presence of alkaloids, flavonoids, saponin, glycosides, quinines, tannins and reducing sugars.

Table 1
Qualitative analysis of Carbohydrates and Secondary metabolites in ethanolic extracts of Emblica officinalis and Solanum nigrum fruits.

S.No.	Phytoconstituents	*E.officinalis*	*S.nigrum*
1.	Phenolic compounds	+	+
2.	Reducing sugars	+	+
3.	Carbohydrates	+	+
4.	Flavonoids	+	+
5.	Glycosides	+	+
6.	Saponins	+	+
7.	Alkaloids	+	+
8.	Quinones	+	+
9.	Tannins	+	+

Table-2
Phytoconsituents identified in ethanolic extract of *S.nigrum* fruit by GC-MS analysis.

S.No.	Peak Name	Retention time	Peak area	% Peak area
1.	2-Cyclopentene-1,4-dione Formula: $C_5H_4O_2$. MW: 96 CAS	6.08	4015464	1.6383
2.	Name: Acetic acid, 2-(dimethylamino)ethyl ester Formula: $C_6H_{13}NO_2$ .MW: 131	6.51	1067665	0.4356
3.	Name: Butyrolactone Formula: $C_4H_6O_2$. MW: 86	6.70	6429523	2.6232
4.	Name: 2-Cyclopenten-1-one, 2-hydroxy- Formula: $C_5H_6O_2$. MW: 98	6.92	2640380	1.0773
5.	Name: 2-Furancarboxaldehyde, 5-methyl- Formula: $C_6H_6O_2$. MW: 110	7.56	1159473	0.4731
6.	Name: 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one Formula: $C_6H_8O_4$.MW: 144	7.84	1766625	0.7208
7.	Name: Glycerin Formula: $C_3H_8O_3$. MW: 92	8.76	112089088	45.7319
8.	Name: 2(3H)-Furanone, dihydro-3-hydroxy-4,4-dimethyl- Formula: $C_6H_{10}O_3$. MW: 130	9.31	4935095	2.0135
9.	Name: 2,5-Dimethyl-4-hydroxy-3(2H)-furanone Formula: $C_6H_8O_3$. MW: 128	9.61	1041045	0.4247
10.	Name: [1,3]Diazepan-2,4-dione Formula: $C_5H_8N_2O_2$ .MW: 128	10.05	1279783	0.5221
11.	Name: 1-Penten-4-one, 2-acetyl-1-dimethylamino- ((Z)- or (E)-) Formula: $C_9H_{15}NO_2$.MW: 169	10.12	3176200	1.2959
12.	Name: 2-Piperidinone, 6-methyl- Formula: $C_6H_{11}NO$. MW: 113	10.45	462079	0.1885
13.	Name: Oxime-, methoxy-phenyl-_ Formula: $C_8H_9NO_2$.MW: 151	10.94	963557	0.3931
14.	Name: 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- Formula: $C_6H_8O_4$.MW: 144	11.34	12586807	5.1354
15.	Name: 4-Hexen-3-ol Formula: $C_6H_{12}O$.MW: 100	11.52	2781176	1.1347
16.	Name: 4-Hexenoic acid, 2,5-dimethyl- Formula: $C_8H_{14}O_2$.MW: 142	11.71	1089646	0.4446
17.	Name: 4H-Pyran-4-one, 3,5-dihydroxy-2-methyl- Formula: $C_6H_6O_4$.MW: 142	11.94	2632634	1.0741
18.	Name: (S)-(+)-2',3'-Dideoxyribonolactone Formula: $C_5H_8O_3$.MW: 116	12.35	756790	0.3088

19.	Name: Piperazine, 1-(aminoacetyl)- Formula: $C_6H_{13}N_3O$.MW: 143	12.44	1209481	0.4935
20.	Name: Cyclohexanemethanol, à-methyl-à-(1-methyl-2-propenyl)-, (R*,S*)-(.+-.)- Formula: $C_{12}H_{22}O$.MW: 182	12.63	3548340	1.4477
21.	Name: Benzofuran, 2,3-dihydro- Formula: C_8H_8O.MW: 120	12.92	8302101	3.3872
22.	Name: 1,6-Heptadiene-4-carboxylic acid, 4-hydroxy-, ethyl ester Formula: $C_{10}H_{16}O_3$.MW: 184.	13.42	10921952	4.4561
23.	Name: Malic Acid Formula: $C_4H_6O_5$.MW: 134	13.90	5428313	2.2147
24.	Name: Naphthalene, 2-methyl- Formula: $C_{11}H_{10}$.MW: 142	14.14	783614	0.3197
25.	Name: 2-Methoxy-4-vinylphenol Formula: $C_9H_{10}O_2$.MW: 150	14.31	2403302	0.9805
26.	Name: 2,4-Dimethylcyclohex-1-ene-5-carboxylic acid (trans) Formula: $C_9H_{14}O_2$.MW: 154	14.91	623837	0.2545
27.	Name: 1(2H)-Naphthalenone, 3,4,4a,5,6,7-hexahydro-6-methyl- Formula: $C_{11}H_{16}O$.MW: 164	14.97	862610	0.3519
28.	Name: 3-Octyne-2,5-dione, 6,6,7-trimethyl- Formula: $C_{11}H_{16}O_2$.MW: 180	15.08	1205554	0.4919
29.	Name: L-Proline, 5-oxo-, methyl ester Formula: $C_6H_9NO_3$.MW: 143	15.56	1686782	0.6882
30.	Name: 1H-Pyrazol-1-amine, 5-methyl-3-nitro- Formula: $C_4H_6N_4O_2$.MW: 142	15.74	1347472	0.5498
31.	Name: 2-Pyrrolidinecarboxylic acid-5-oxo-, ethyl ester Formula: $C_7H_{11}NO_3$.MW: 157	16.56	6838759	2.7902
32.	Name: dl-4-Amino-3-hydroxybutyric acid Formula: $C_4H_9NO_3$.MW: 119	16.76	7930810	3.2357
33.	Name: 2-Pyrrolidinone, 1-butyl- Formula: $C_8H_{15}NO$.MW: 141	17.65	1974094	0.8054
34.	Name: 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl- Formula: $C_{11}H_{16}O_2$.MW: 180	18.12	570940	0.2329
35.	Name: Ethanone, 1-(3,4-dimethoxyphenyl)- Formula: $C_{10}H_{12}O_3$.MW: 180	18.38	1104094	0.4505
36.	Name: 3-Hexadecene, (Z)- Formula: $C_{16}H_{32}$.MW: 224	18.46	1067752	0.4356
37.	Name: Ethyl N-(2-methylphenyl)carbamate Formula: $C_{10}H_{13}NO_2$.MW: 179	19.06	812105	0.3313
38.	Name: 2',4'-Dihydroxy-3'-methylbutyrophenone Formula: $C_{11}H_{14}O_3$.MW: 194	19.14	398577	0.1626
39.	Name: 1H-Indole-2-carboxylic acid, 5-chloro-, ethyl ester. Formula: $C_{11}H_{10}ClNO_2$.MW: 223	19.36	1253655	0.5115
40.	Name: Ethyl á-d-riboside Formula: $C_7H_{14}O_5$.MW: 178	19.46	1404456	0.5730
41.	Name: Ethyl à-d-glucopyranoside Formula: $C_8H_{16}O_6$.MW: 208	19.64	1404503	0.5730
42.	Name: Z-2-Dodecenol Formula: $C_{12}H_{24}O$.MW: 184	20.34	1146405	0.4677
43.	Name: 1,1'-Biphenyl, 2,2',5,5'-tetramethyl- Formula: $C_{16}H_{18}$.MW: 210	20.47	825364	0.3367
44.	Name: Propionic acid, 3-(m-aminobenzoyl)-2-methyl- Formula: $C_{11}H_{13}NO_3$.MW: 207	20.89	2282230	0.9311
45.	Name: Tetradecanoic acid Formula: $C_{14}H_{28}O_2$.MW: 228	21.09	2437556	0.9945
46.	Name: Benzenemethanol, 2-hydroxy-5-methoxy-à,à,3-trimethyl-. Formula: $C_{11}H_{16}O_3$.MW: 196	21.77	2023717	0.8257
47.	Name: Benzoic acid, 3-amino-, ethyl ester Formula: $C_9H_{11}NO_2$.MW: 165	21.88	2353133	0.9601
48.	Name: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol Formula: $C_{20}H_{40}O$. MW: 296	21.94	3177117	1.2963
49.	Name: 2-Pentadecanone, 6,10,14-trimethyl- Formula: $C_{18}H_{36}O$.MW: 268	22.08	1395381	0.5693
50.	Name: 1,6-Octadiene, 2,5-dimethyl-, (E)- Formula: $C_{10}H_{18}$.MW: 138	22.62	3001612	1.2246
51.	Name: 4(1H)-Pyrimidinone, 6-amino-2-methyl-5-nitroso- Formula: $C_5H_6N_4O_2$.MW: 154	24.04	2501540	1.0206

The phytoconstituents such as butyrolactone; 2-Cyclopentene-1,4-dione; 2-Cyclopenten-1-one 2-hydroxy; 2-Piperidinone; 6-methyl-, 2-Pyrrolidinone; 1-butyl-, phenol etc., were found to be present in

the ethanolic extract of *S. nigrum* fruits. Similarly, *E.officinalis* fruits extract found to possess hexadecanoic acid; methyl ester; 2, 4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one; Benzenecarboxylic acid; Malic Acid; Octadecanoic acid, methyl ester etc.

Table 3

Phytoconsituents identified in ethanolic extract of E. officninalis fruit by GC-MS analysis,

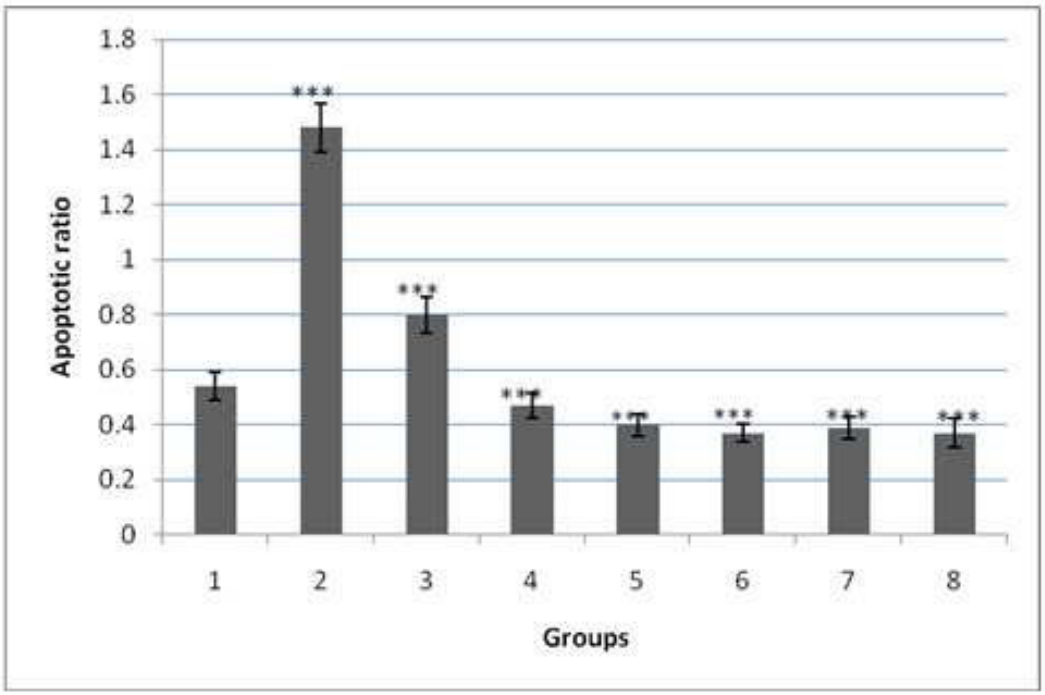
S.No.	Peak Name	Retention time	Peak area	% Peak area
1.	Name: 2-Amino-oxazole Formula: $C_3H_4N_2O$.MW: 84	4.75	3879351	0.2245
2.	Name: 1,2-Cyclopentanedione Formula: $C_5H_6O_2$.MW: 98	4.93	1553010	0.0899
3.	Name: 2,5-Furandione, dihydro-3-methylene- Formula: $C_5H_4O_3$ .MW: 112	5.17	3607132	0.2087
4.	Name: Methyl 2-furoate Formula: $C_6H_6O_3$.MW: 126	5.64	426991	0.0247
5.	Name: 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one Formula: $C_6H_8O_4$ .MW: 144	5.72	6386979	0.3695
6.	Name: Phenol Formula: C_6H_6O.MW: 94	5.94	1017556	0.0589
7.	Name: 2(5H)-Furanone Formula: $C_4H_4O_2$.MW: 84	6.13	105114232	6.0818
8.	Name: 1H-Pyrrole, 2,5-dihydro- Formula: C_4H_7N.MW: 69	6.57	1915953	0.1109
9.	Name: 4-Methoxycarbonyl-4-butanolide.Formula: $C_6H_8O_4$.MW: 144	8.00	32311438	1.8695
10.	Name: 4-Heptanone, 2,6-dimethyl- Formula: $C_9H_{18}O$. MW: 142	8.39	1288369	0.0745
11.	Name: 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-Formula: $C_6H_8O_4$. MW: 144	8.84	31224788	1.8066
12.	Name: Benzenecarboxylic acid Formula: $C_7H_6O_2$.MW: 122	9.28	3571438	0.2066
13.	Name: 4H-Pyran-4-one, 3,5-dihydroxy-2-methyl- Formula: $C_6H_6O_4$MW: 142	9.56	1525841	0.0883
14.	Name: Pyrrolin-2-one-5-methanol, N-methyl- Formula: $C_6H_{11}NO_2$ .MW: 129	10.00	921567	0.0533
15.	Name: Propanoic acid, 2-methyl-, pentyl ester Formula: $C_9H_{18}O_2$.MW: 158	10.19	5372068	0.3108
16.	Name: Malic Acid Formula: $C_4H_6O_5$.MW: 134	11.12	64700960	3.7435
17.	Name: Ethanone, 1-(2-hydroxy-5-methylphenyl)- Formula: $C_9H_{10}O_2$.MW: 150	11.74	3340164	0.1933
18.	Name: Benzaldehyde, 2-hydroxy-4-methoxy- Formula: $C_8H_8O_3$.MW: 152	12.13	4451325	0.2575
19.	Name: 1,2,3-Benzenetriol Formula: $C_6H_6O_3$ .MW: 126 Pyrogallol	13.58	1321102336	76.4370
20.	Name: D-Allose Formula: $C_6H_{12}O_6$.MW: 180	15.79	96451064	5.5805
21.	Name: 1,6-Anhydro-á-D-glucofuranose Formula: $C_6H_{10}O_5$.MW: 162	17.47	32437078	1.8768
22.	Name: Hexadecanoic acid, methyl ester Formula: $C_{17}H_{34}O_2$.MW: 270	20.83	2720290	0.1574
23.	Name: 9,12-Octadecadienoic acid, methyl ester Formula: $C_{19}H_{34}O_2$.MW: 294	24.56	1075011	0.0622
24.	Name: 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- Formula: $C_{19}H_{32}O_2$.MW: 292	24.71	1259281	0.0729
25.	Name: Octadecanoic acid, methyl ester Formula: $C_{19}H_{38}O_2$.MW: 298	25.25	699845	0.0405

Apoptotic ratio

In the present study, the apoptosis modulating effects of the fruit extracts were studied using oxidants namely H_2O_2.The morphological changes of apoptosis observed in yeast cells were quantified in all the experimental groups. The numbers of apoptotic cells or the apoptotic ratio in each experimental group is represented graphically (Figure 1)

Figure-1

Apoptotic ratio of yeast cell of different experimental groups.

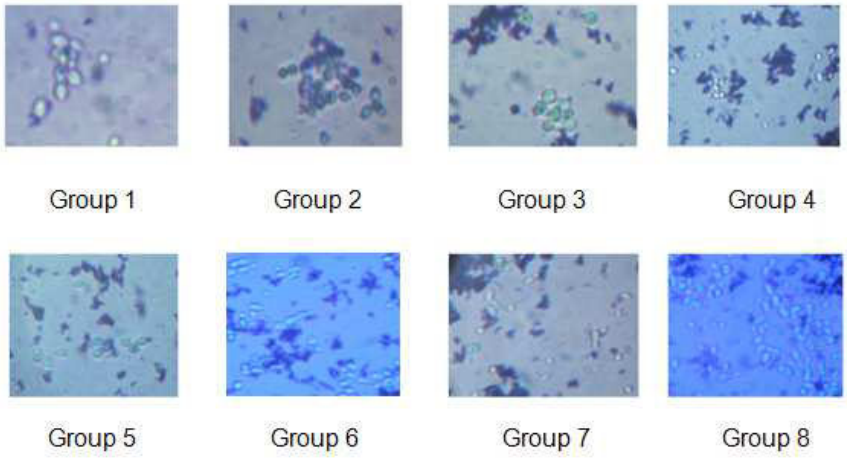


Values are expressed as Mean ± SD for six experiments. P <0.001 Group 2 with Group 1 and Group 3 to 8 with Group 2***

The cells treated with H_2O_2 showed well defined apoptotic morphology and were identified with loss of membrane integrity, cell shrinkage and apoptotic blebbing (Plate 1). The rate of cells undergoing apoptosis was strongly hindered by the treatment with the fruit extracts compared to H_2O_2 alone treated group. Among the two fruit extracts anti-apoptotic effect was found to be more in the group enriched with 50 µg/ml fruit extract of *S. nigrum*. Vitamin E has higher apoptosis inhibiting effect than the plant extracts.

Plate-1

Morphological changes in S.cerevisiae cells of experimental groups subjected to oxidative stress (Giemsa staining) (2300X magnification)

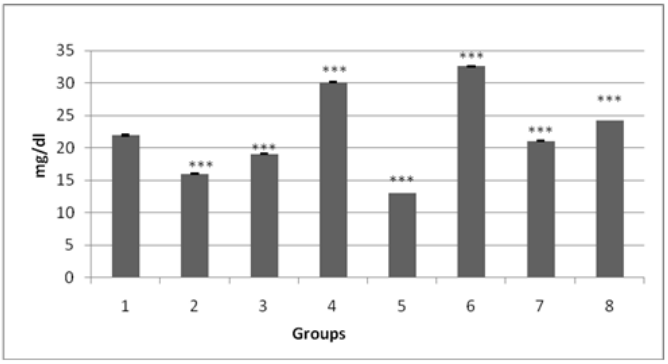


Protein level

Level of protein in fruit extract treated *S. cerevisiae* is represented in Figure 2. The level of protein in H_2O_2 treated yeast cell (group 2) was found to be reduced as compared to control cells. Protein level in *S. nigrum* fruit extract treated group (50µg/ml) showed a marked increase as compared to group 2, followed by the group treated with fruit extract of *E.officinalis* with same concentration. The groups with two different concentrations of vitamin E also showed significant increase in protein level when compared to the group treated with H_2O_2.

Figure-2

Level of protein in the yeast cell extract of different groups. (Values are expressed as mean ± S.D (n=6)).



Values are expressed as Mean ± SD for six experiments, P <0.001 Group 2 with Group 1 and Group 3 to 8 with Group 2***

Antioxidant enzymes activity in S. cerevisiae

Activity of catalase and super oxide dismutase showed marked decrease as compared to control yeast cell extract (table 4), where as either the yeast cells pretreated with plant cell extract or vitamin E showed significant increase in the activity as compared with treated with H_2O_2 alone. Of the different yeast groups, yeast cells treated with 50µg/ml of *S.nigrum or E. officinalis* extract showed almost twofold increase as compared with the cells treated with 25µg/ml fruit extract. Similarly, the groups treated with vitamin- E also showed increased activity in dose dependent manner.

Table 4
Activity of Catalase and Super oxide dismutase in S.cerevisiae.

Groups	Catalase*	SOD*
Group 1	31.60 ± 2.67	20 ± 1.67
Group 2	5.09 ± 0.33$***	4 ± 0.023$***
Group 3	15.49 ± 0.86#***	16 ± 1.14#***
Group 4	28.91 ±1.80#***	24 ± 1.85#***
Group 5	12.70 ±0.98#***	12 ± 0.89#***
Group 6	45.87 ±3.30#***	20 ± 1.27#***
Group 7	9.98 ±0.83#***	16 ± 0.97#***
Group 8	21.26 ±1.21#***	20 ± 1.32#***

Units/mg protein Values are expressed as Mean ± SD for six experiments
P <0.001 $ Group 2 with Group 1 and # Group 3 to 8 with Group 2***

DISCUSSION

Antioxidants from plants with free radical scavenging ability are gaining importance as therapeutic agents in oxidative stress related disease. Active principles and phytomedicines are still used by more than 75% of the world population especially in developing countries for acceptability and better compatibility with human body[14]. GC-MS analysis of the fruit extract of *S.nigrum* and *E.officinalis* revealed the presence of several phytoconstituents (Table 2 and 3). Most of these compounds were reported to have antioxidant, antimicrobial and anti-tumor activity. (Dr. Duke's phytochemical and ethnobotanical databases) Phenolics compounds are food candidates as antioxidants because of their favourable potentials and the relative stability of the redox radical [15], the compounds such as flavonoids and tannins act as primary free radical scavengers[16]. Nuclear fragmentation, chromatin condensation, shrinkage of cytoplasm and formation of apoptotic bodies are the index of apoptotic cell death [17]. From the results of Giemsa staining (plate 1), it is observed that the cytoprotection activity of these fruits extract is dose dependent, the apoptotic ratio was higher in the yeast cells treated with 25µg/ml (group 3) than the group treated with 50µg /ml of *S.nigrum* fruit extract. But there was no marked dose dependent difference in apoptotic ratio in yeast cells treated with *E.officinalis* fruit extract. A significant increase in apoptotic ratio was noticed in group 2 as compared with the groups treated with fruit extracts or vitamin-E treated groups. The decrease in apoptotic ratio or cytoprotective activity could be due presence of various secondary metabolites in the extract. Phytoconstituens such as ellagitannins and propelargonidin isolated from *Syzygium cumini* fruit, flavonoids, glucosides like compounds from aerial parts of *Verbascum salviifolium,* alkaloid from *Fumaria capreolata* and protopine, cryptonine, stylopine, dumariline, phtalidusoquinoline, fumaritine, fumarafine and dehydrobenzophenantridine *Fumaria bastardii* were reported to possess antioxidant activity[14]. Antioxidants exert their activity through several mechanisms such as by the inhibition of active species formation, reduction of hydrogen peroxides, hydroperoxides, eliminating reactive free radicals and also by repairing or clearing damage of cell[18]. Oxidative stress is the presence of excess of reactive oxygen species (ROS) than antioxidants, these ROS can damage proteins, DNA, lipids and thereby alter the organism's structure and functions. On

administration of H_2O_2 free in yeast cell leads the formation free radical and which in turn leads the apoptosis, as it was observed from the group 2. As the fruit extract has several phytoconsituents they could combat the oxidative stress induced by H_2O_2 as it was evident from the observation of the yeast cells (groups 3-6) pretreated with the fruit extract. It is also evident that the level of protein in group 2 was found to be decreased as compared with control, whereas the yeast cells pre-treated with fruit extract retained their protein level to near normal level (Figure 2). The compounds such as Butyrolactone, 2-Piperidinone, 6-methyl-, 2-Pyrrolidinone, 1-butyl-, Phenol and Hexadecanoic acid, methyl ester that present in the fruit extracts could scavenge the free radical and protect of proteins in the yeast cells that undergone oxidative stress induced by H_2O_2. SOD is an important antioxidant enzyme, which is considered as the main defense system as they scavenge the reactive oxygen species and forms H_2O_2, catalase in turn decomposes H_2O_2 thereby reducing the oxidative stress. As these two enzymes play an important role in alleviating the oxidative stress their activity was determined. Previous reports revealed that oral administration of an aqueous extract of *Annona squamosa* leaf combated the streptozotocin induced oxidative stress by increasing enzymic antioxidants like SOD, catalase and glutathione peroxidase activities[19] and crude extract of mango in Swiss albino mice restored the activities of antioxidant enzymes (SOD and catalase)[20]. Radha *et al.,* had shown that liver slices exposed with H_2O_2 showed significant reduction of all the antioxidants, which was effectively reverted by the administration of *M.hortensis* leaf extract [21]. Our results are in accordance with the previous reports that administration of fruit extract elevated the activities of these antioxidant enzymes.

CONCLUSION

Globally there has been growing interest to identify herbal antioxidants which are pharmacologically potent and have less or no side effects. On the basis of our results presented here we propose that the fruit extracts of *S.nigrum* and *E.officinalis* have protective effect against H_2O_2 induced oxidative damage in *S.cerevisiae.* Among the two fruit extracts, *S.nigrum* showed higher protective effect than *E.officinalis* fruit extract against oxidative stress. Hence the plant based products or phytoconstituents could be used to treat the ROS mediated diseases.

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