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J. Nat. Prod. Plant Resour**., 2012, 2 (1):192-197**
(http://scholarsresearchlibrary.com/archive.html)

ISSN : 2231 – 3184
CODEN (USA): JNPPB7

Study of antioxidant and anticancer activity of natural sources

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ABSTRACT

The use of Synthetic antioxidants is been widely restricted because of their reported toxic and carcinogenic effects. Thus now there is considerable interest being focused towards finding antioxidants from natural sources with any unlikely effects. There is also considerable evidence which indicates lower risk of Cancer in people with high intake of fruits and vegetables, which is presumingly because of the antioxidant or other active compounds present in them. The present study was conducted to determine antioxidant activity of Streptomyces sps.PDS1,commercially available Red Wine and Saccharomyces cerevisiae MTCC-181 using the Ferric reducing assay and to study the anticancer activity of Green Tea and Citrus limetta against human lung carcinoma cell line A549 using the MTT assay.The isolates of Streptomyces sps.PDS1 was found to have more antioxidant activity Saccharomyces cerevisiae.The antioxidant potential was then correlated with the total phenol and flavonoid content.The filtrates of Green Tea showed maximum anticancer activity against cancer cell line A549 as compared to that of Citrus limetta.

Key Words: Antioxidants, *Streptomyces* PDS1, *Saccharomyces cerevisiae,* MTT assay, Anticancerous activity.

INTRODUCTION

An Antioxidant is a molecule capable of inhibiting the oxidation of other molecules.Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions[1].

Relatively strong reducing acids can have antinutrient effects by binding to dietary minerals such as iron and zinc in the gastrointestinal tract and preventing them from being absorbed.[2].Notable examples are oxalic acid, tannins and phytic acid, which are high in plant-based diets.[3]. Cocoa bean and chocolate, spinach, turnip have high amounts of Oxalic acid[4].Whole grains, maize, legumes have notable amounts of Phytic acid[5]. Tea, beans, cabbage also have high amounts of Tannins[6].Polyphenolic antioxidants (resveratrol, flavonoids) are found in coffee, soy, fruit, olive oil, chocolate, cinnamon, oregano. Vitamin E (tocopherols, tocotrienols) is present in Vegetable oils. Glutathione is a cysteine-containing peptide has antioxidant properties since the thiol group in its cysteine moiety is a reducing agent and can be reversibly oxidized and reduced[7].In some organisms glutathione is replaced by other thiols, such as by mycothiol in the Actinomycetes, or by trypanothione in the Kinetoplastids[8].

Treating cells with a cytotoxic compound can result in a variety of cell fates. The cells may undergo necrosis, in which they lose membrane integrity and die rapidly as a result of cell lysis. The cells can stop actively growing and dividing (a decrease in cell viability), or the cells can activate a genetic program of controlled cell death (apoptosis). Assessing cell membrane integrity is one of the most common ways to measure cell viability and cytotoxic effects[9].

MATERIALS AND METHODS

Sample Collection:
The samples for Green Tea, Citrus limetta & Red Wine were collected from different areas of Coimbatore .*Streptomyces* sps.PDS1 was procured from Department of Biotechnology, SNMV CAS, Coimbatore, India. *Saccharomyces cerevisiae* MTCC 181 was obtained from IMTECH, Punjab. Human lung carcinoma cell line A549 was collected from National Centre For Cell Science, Pune,India.

Preparation of Methanolic extract
Methanolic extract was prepared according to the method described by Farrukh [14] . 10 g of Green Tea & Citrus limetta fruit pulp was weighed and soaked in 32 ml of 98 % methanol for 5 days. The extract was then filtered using Whatman filter paper No.1 and the filtrate obtained was collected for further studies

Preparation of Crude sample from *Streptomyces* PDS1
The Crude sample was prepared according to the method given by Prashith [11]. Starch casein broth of volume 50 mL was prepared(pH = 7.2).The culture was inoculated into Starch casein broth which prepared and then the flask was kept in shaker. After 7 days,the broth was centrifuged and the pellet was used for further studies.

Preparation of Crude sample from *Saccharomyces cerevisiae*
The Crude sample was prepared according to the method given by Prashith [11] The culture was inoculated in 50mL of Yeast peptone dextrose broth (pH= 7.0) and then the flask was kept in shaker. After incubation for 24 h, the inoculated broth was centrifuged and the pellet was collected as crude sample which was used for further studies.

Ferric reducing assay

The amount of Antioxidant present was determined using the method described by Prashith [11] Two concentrations, namely 0.5 and 0.5 mg/mL, of solvent extracts and tannic acid in 1ml of methanol were mixed in separate tubes with 2.5mL of phosphate buffer (200mM, pH=6.6) and 2.5ml of 1 % potassium ferricyanide. The mixture was placed in a water bath for 20 min at 50°C, cooled rapidly and mixed with 2.5mL of 10 % trichloroacetic acid and 0.5mL of 0.1 % Ferric chloride.The amount of iron (II)- ferricyanide complex formed was determined by measuring the optical density at 700nm after 10min.The higher absorbance of the reaction mixture indicates increased reducing power.

Total phenol content

The Colorimetric method described by Guleria[12] was used. Sample extract of 0.5 mL was added to 0.5 mL of 1N Folin-Ciocalteu reagent. The mixture was kept at room temperature, followed by the addition of 1 mL of 20 % Na_2CO_3. After 10 min of incubation at room temperature, the absorbance was read at 730 nm using UV-VIS spectrophotometer

Flavonoid content

The Flavonoid content was estimated using the Colorimetric method [12]. Plant extracts (250µl) was mixed with 1.25 mL of distilled water & 75 µl of 5 % $NaNO_2$ solution. After 5 min,150 µl of 10 % $AlCl_3$ was added. After 6 min,500 µl of 1M NaOH & 275 µl of distilled water were added to prepare the mixture.The solution was mixed well & the absorbance was read at 510 nm using UV-VIS spectrophotometer.

Cytotoxicity determination by MTT assay

A549 cancer cells were sub cultured in DMEM media supplemented with 2mM L-glutamine adjusted with 1.5g/L Sodium bicarbonate and 90% fetal calf serum incubated at 37ºc in 5% CO_2 incubator.Different concentrations of Green Tea & Citrus limetta filtrate(20-100µl) were added to the A549 cancer cells(6 µl),seeded in 96-well microtiter & incubated at 37ºc for 24 hours.At the end of the treatment,20 µl of MTT [(3,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)] was added to each well & the microtiter plate were incubated for 4hrs at 37ºc.Finally, acidic isopropanal (100 µl) was added to each well, after which optical absorbance was read at 595nm on multi well spectrophotometer plate reader.

The percentage viability was calculated as follows:

Cell Viability = Optical density of samples/Optical density of control X 100.

RESULTS AND DISCUSSION

Free radicals are chemical species containing one or more unpaired electrons that makes them highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability[17].Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS [18]. In recent years much attention has been devoted to natural antioxidant and their association with health benefits . There are several methods available to assess antioxidant activity of compounds[17].

An easy, rapid and sensitive method for the antioxidant screening is the Ferric reducing assay. The reducing capacity of compound may serve as significant indicator of its potential antioxidant activity .The antioxidant activities have been reported to be the concomitant development of reducing power [15]. In the present study, the antioxidant activity was found to be maximum in commercial Red Wine followed by *Streptomyces* sps.PDS1 and *Saccharomyces cerevisiae* MTCC-181(Table.1) .The results were correlated to the findings of antioxidant activity of two Streptomyces species isolated from Western Ghat soils of Agumbe, Karnataka.[11]

Table 1. Antioxidant activity of *Streptomyces sps* PDS1

Concentration (mg/ml)	Absorbance at 700 nm			
	Standard(Tannic Acid)	*Streptomyces sps* PDS1.	*Saccharomyces cerevisiae*	Red Wine
0.5	0.957	-	-	-
0.5	-	0.569	0.450	0.574

The free radical scavenging activity of crude samples used may be attributed to the presence of phenolic compounds as these compounds exhibit important mechanism of antioxidant activity[15].Antioxidant activity of phenolic compounds is probably due to their redox properties,which allow them to act as reducing agents, singlet oxygen quenchers, metal ion chelators and hydrogen donors [16].In all the three natural sources tested,the highest phenol content was found to be in Red Wine followed by *Streptomyces* sps.PDS1 and then *Saccharomyces cerevisiae*(Table 2,3). The maximum flavonoid content was found to be in *Saccharomyces cerevisiae* followed by *Streptomyces* sps. PDS1 and Red Wine(Table 4,5) .The results were correlated to the total phenol content in Terminalia bellerica Roxb [15], Trigonella foenum-graecum [10].

Table 2.Total phenol content

Sample used	Red wine*	*Streptomyces Strain C#*	*Saccharomyces cerevisiae#*
O.D at 730 nm	0.380	0.115	0.320

**-0.5 ml , # - 0.5 mg of pellet used.*

Table 3.The Phenol content

Samples used	Red Wine	*Streptomyces Strain C*	*Saccharomyces cerevisiae*
in mg/l	630	170	540

The protective effect of fruits and vegetables with respect to anticancer activity is assumed to be associated mainly with the antioxidant activities of either individual or interacting bioactive components present in the fruits and vegetables, and with other biochemical and physical characteristics of the identified and unknown bioactive components. The implicated bioactive components present in citrus fruits include vitamin C, β-carotene, flavonoids, limonoids, folic acid, and dietary fibre. A high intake of citrus fruits may reduce the risk of degenerative diseases [13].

Table 4.Total flavonoid Content

Samples used	Red wine*	*Streptomyces Strain C#*	*Saccharomyces cerevisiae#*
O.D at 510 nm	0.157	0.441	0.375

**-0.5 ml , # - 0.5 mg of pellet used.*

Table 5. The Flavonoid content

Samples used	Red Wine	*Streptomyces Strain C*	*Saccharomyces cerevisiae*
in µg/ml	4	12	10

When the cancer cell line was incubated with A549 the presence of non viable cells was found. The cell viability was determined by MTT Assay. Different concentrations of Green Tea & *Citrus limetta* extracts were added to the cancer cell lines & the anticancer activity was detected.(Table 6).

Table 6.Anticancerous activity of Green Tea and *Citrus limetta*(Fig.1)

Concentration (in µl)	Control	20	40	60	80	100
Green Tea	0.474	0.367	0.656	0.826	0.950	0.962
Citrus limetta	0.129	0.080	0.106	0.109	0.115	0.123

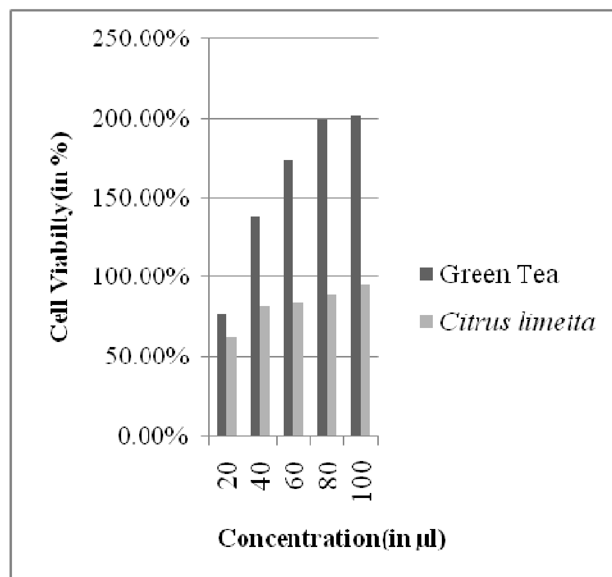

Fig.1 Comparative study of anticancer activity of Green Tea and *Citrus limetta*.

CONCLUSION

Based on the reported results,it can be concluded that along with Red Wine,even microorganisms *Streptomyces* and *Saccharomyces cerevisiae* have potential antioxidant activity.The anticancerous activity of Green Tea was found to be more than that of *Citrus limetta* ,thus implicating its use in prevention and cure of Cancer.

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