Food Sci. Biotechnol. 20(1): 7-13 (2011)
DOI 10.1007/s10068-011-0002-0

RESEARCH ARTICLE

Evaluation of Antibacterial, Antifungal, and Antioxidant Properties of Some Food Dyes

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Received: 1 June 2010 / Revised: 2 October 2010 / Accepted: 21 October 2010 / Published Online: 28 February 2011

Abstract Natural dyes find use in the coloring of textiles, drugs, cosmetics, etc. Owing to their nontoxic effects, they are also used for coloring various food products. In the present study antimicrobial properties of 8 food dyes against 10 bacteria and 5 fungal organisms were investigated. It was observed that red dyes showed best antibacterial activity while yellow dyes showed better antifungal activity. Dyes obtained from catechu (*Acacia catechu*) and myrobalan (*Terminalia chebula*) is not sufficiently effective against the tested microorganisms. In addition to antimicrobial analysis, antioxidant activity by 3 different methods was also investigated. In all the methods, red dye was found to have greater antioxidant activity. It suggest that the addition of these dyes in food not only enhances the value addition by making the food more presentable but also shall address the issue of food supplementation with substances that are good antibiotics and antioxidants, subsequently proving to be health benefactors.

Keywords: food dye, antibacterial, antifungal, antioxidant

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Introduction

Color in one form or other, has been added to our foods for centuries. There has been much interest in the development of new natural colorants for use in the food industry, which is apparently due to strong consumer demand for more natural products, at least in some countries. There is no doubt that it is technologically feasible to prepare new natural colorants from locally known plants or microorganisms that have not yet been investigated scientifically (1). Currently, 43 colorants are authorized as food additives by the Council of the European Union, and have been assigned an E number. Sixteen of these are of plant origin. Juices or extracts from some fruit and vegetables are also used for coloring purposes (2).

Natural products have been used in traditional medicine through out the world and predate the introduction of antibiotics and other modern drugs. The antimicrobial efficacy attributed to some plants or compounds in treating diseases has been beyond belief. It is estimated that local communities have used about 10% of all flowering plants on earth to treat various infections, although only 1% have gained recognition by modern scientists (3). Owing to their popular use as remedies for many infectious diseases, searches for plants containing antimicrobial substances are frequent. Thus the hunt for novel plant based product should thus be a priority in current and future efforts toward sustainable conservation and rational utilization of nature (4). Nowadays, fortunately, there is an increasing awareness among the people towards the use of natural dyes as substitute for synthetic dyes. Due to its non-toxic property, low pollution, and less side effects, natural dyes are used more often in food products as well as other important regular uses. Above all, they are environment-friendly and after use, can be recycled (5). There are

Table 1. Details of the selected dyes in food sector

Name of the plant	Common name of the dye	Uses in food sector
Acacia catechu (L.f) Willd	Catechu	Used as a food additives. In south India (esp. Kerala) wood is boiled in drinking water and treated as beverages. The dye also used as a flavor in the preparation of sauce (22).
Bixa orellana L.	Annatto	The dye is used as a colorant for rice, cheese, and sweets. Grounded seeds are used as condiments (23).
Cassia auriculata L.	Senna dye	The yellow dye is generally added as a flavor in coffee or tea preparation. It is classified as famine food and leaves are generally edible (6).
Embilica officinalis Gaertn.	Amla dye	Fruit is commonly used as an antioxidant. Juice obtained from the fruit is used as a health drink rich in vitamin C (24).
Punica granatum L.	Pomegranate	Fruit and its juice are widely consumed. The dried fruits are used as spices (6).
Terminalia chebula Retz.	Myrobalan	Food additive, one of the constituents of 'triphala', an important ayurvedic medicine for cleansing and detoxifying the body (25).
Tagetes erecta L.	Marigold dye	Dye can be used as a saffron substitute for coloring and flavoring in foods and tobacco industry (25).
Rubia tinctorum L.	Madder dye	Used as a flavoring agent and food additive (23).

several studies revealing that some of the dye yielding plants also possesses medicinal properties (6).

In developing countries like India people use folk medicine for the treatment of common infections. These plants are ingested as decoctions, teas, and juice preparations to treat various infections. It is necessary to evaluate, in a scientific base, the potential use of folk medicine for the treatment of infectious diseases produced by common pathogens. Medicinal plants or natural product might represent an alternative treatment in non-severe cases of infectious diseases (7). They can also be a possible source for new potent antibiotics to which pathogen strains are not resistant. Owing to their popular use as remedies for many infectious diseases, searches for plants containing antimicrobial substances are frequent. According to the World Health Organization, medicinal plants would be the best source for obtaining variety of drugs (8). These evidences contribute to support and quantify the importance of screening natural products. Although known for a long time for dyeing as well as medicinal properties, the structures and protective properties of natural dyes have been recognized only in the recent past. Many of the plants used for dye extraction are classified as medicinal, and some of these have recently been shown to possess remarkable antimicrobial activity (9). The aim of the present study was to investigate the antibacterial and antioxidant properties of some food dyes. Some of the natural dyes that have been routinely used in the food and other allied fields are mentioned in the Table 1. Evidently, as there are not sufficient scientific studies that confirm the antimicrobial and antioxidant properties of these dyes, the present investigation is a valid one.

Material and Methods

Food dyes The optimized natural dye extraction protocol was carried out on the following 8 dye yielding plants: *Acacia catechu* L., *Bixa orellana* L, *Cassia auriculata* L., *Embillica officinalis* Gaertn, *Punica granatum* L., *Rubia tinctorum* L, *Tagetes erecta* L., and *Terminalia chebula* Retz. All the plant materials were collected from the vicinity of VIT University Campus, Vellore, Tamil Nadu, India. The details on the above mentioned plants were described in Table 2.

Test organism Cultures of the following 15 micro-organisms consisting of 10 bacteria viz., *Bacillus subtilis, Staphylococcus aureus, Vibrio fischeri*, *Yersinia enterocolitica*, *Proteus vulgaris*, *Lactobacillus* sp., *Lactococcus* sp., *Pediococcus pantosaceus*, *Staphylococcus* sp., *Pseudomonas* sp., and 5 fungal organisms *Candida albicans*, *Candida famata*, *Rodotorula*, *Aspergillus* sp., and *Neurospora crassa* were used in the study. Table 3 shows details on test organisms. Organisms were procured from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, Punjab, India. All chemicals and reagents for the experiments were of analytical grade and purchased from Sigma-Aldrich, Bangalore, India.

Determination of anti-microbial test Nutrient agar medium (g/L: peptone 5.0; beef extract 1.5; yeast extract 1.5; NaCl 5.0; agar 20; pH 7.5) was prepared and autoclaved at 121°C for 20 min. Sterilized petri plates were prepared with an equal thickness of nutrient agar. Test organisms were grown overnight at 37°C, 120 rpm in 10

Table 2. Details on the natural dyes used in the present study

Plant	Family	Part used	Color obtained	Pigment	Chemical group	Other use
Acacia catechu (L.f.) Willd	Mimosaceae	Bark	Brown	Catechin, catechutanic acid	Flavone-3-ol	Cure skin diseases
Bixa orellana L.	Bixaceae	Seed	Red	Bixin	Apocarotenoid	Astringent, skin disorders, dysentery, hepatitis, elimination of phlegm in new borns, antivenom for snakebites
Cassia auriculata L.	Caesalpineaceae	Flower	Yellow	ND	Anthraquinone	Leaf decoction used to arrest thirst during illness
Embillica officinalis Gaertn.	Phyllanthaceae	Fruit	Black	ND	Polyphenol	Making the body cool, very good hair tonic
Punica granatum L.	Lythraceae	Fruit	Red	Petargonidon 3,5 diglucoside	Anthocyanin	Fruits edible
Rubia tinctorum L.	Rubiaceae	Root	Red	Alizarin	Anthraquinone	Jaundice, obstruction of the spleen, melancholy, palsy, haemorrhoids, sciatica, bruises
Tagetes erecta L.	Asteraceae	Flower	Yellow	Lutein	Xanthophyll	In treating espanto, a typically ill-defined Andean pathology with psychosomatic problems
Terminalia chebula Retz.	Combretaceae	Bark	Brown	Chebulinic acid	Ellagitannin	Fruit-cough, asthma, black dye, indigestion, medicine, seeds-wounds

Table 3. Details of the microorganisms used

Organism name	MTCC No.	Family	Pathogenicity
(A) Gram-positive bacterial strains			
Bacillus subtilis	441	Bacillaceae	Non-pathogenic
Lactobacillus sp.	2,997	Lactobacillaceae	Non-pathogenic
Lactococcus sp.	401	Streptococcaceae	Non-pathogenic
Pediococcus pantosaceus	3,817	Lactobacillaceae	Non-pathogenic
Staphylococcus aureus	24	Saccharomycetaceae	Pathogenic
Staphylococcus sp.	2,940	Saccharomycetaceae	Pathogenic
(B) Gram-negative bacterial strains			
Proteus vulgaris	1,771	Enterobacteriaceae	Pathogenic
Pseudomonas sp.	7,296	Pseudomonads	Pathogenic
Vibrio fischeri	111	Vibrionaceae	Non-pathogenic
Yersinia enterocolitica	840	Enterobacteriaceae	Pathogenic
(C) Fungal strains			
Aspergillus sp.	2,669	Trichocomaceae	Pathogenic
Candida albicans	227	Saccharomycetaceae	Pathogenic
Candida famata	3,279	Saccharomycetaceae	Pathogenic
Rodotorula sp.	3,353	Saccharomycetaceae	Pathogenic
Neurospora crassa	260	Sordariaceae	Pathogenic

mL nutrient broth. This broth was used for seeding the agar plates. A different concentration of each dye was impregnated onto a small disc of filter paper (diameter 5.0-mm) and placed on top of the seeded medium. After overnight incubation at 37ºC, the zones of inhibition were measured and compared with the zones obtained from a standard commercially available antibiotic disc.

Antioxidant assay Antioxidant activity was measured by 3 different method viz., 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, β-carotene bleaching assay, and ferric reducing antioxidant power (FRAP) assay.

DPPH free radical scavenging activity The ability of the extract to scavenge DPPH radicals was assessed as described by Shimada *et al*. (10) and Yang *et al*. (11). Accordingly, 0.5 mL aliquot containing different concentrations of dyes, 0.5 mL of water control was mixed with 3 mL of freshly prepared ethanolic DPPH (0.1 mmol/L) solution. After 30 min of incubation in dark, the absorbance was recorded at 517 nm. Butylated hydroxy toluene (BHT) was used as a positive control and the experiment was performed for 2 times. Percentage inhibition of the radicals due to the antioxidant property of the dyes was calculated using the formula

$$\% \text{ Inhibition} = [1 - (Abs_{sample}/Abs_{control})] \times 100$$

The effective concentration or EC_{50} values were calculated from the graph using the software Graph Pad Prism (Hearne Scientific Software, Melbourne, Australia).

β-Carotene bleaching assay This was performed as per Graven *et al*. (12) method. Linoleic acid solution (10 mL of 2 mg/mL solution in ethanol) and β-carotene solution (10 mL, 2 mg/mL solution in acetone) were added to the molten agar (10 mL, 1.2% solution in boiling water). The mixture was then shaken to give an orange color. The agar was then poured into petri dishes (25 mL/dish) and was excluded from light and left standing to allow the agar to set. Holes (4-mm diameter) were then punched into the agar and each extract (1 mg) in dimethyl sulfoxide (DMSO) were transferred into the holes and the petri dishes were then incubated at 45ºC for 4 hr. A zone of color retention around the hole after incubation indicated sample with antioxidant activities. Ascorbic acid was used as a standard and experiment was performed 2 times. The zone diameter was measured.

FRAP assay The reducing power was determined according to the method of Oyaizu (13). One mL of the dye sample (1 mg/mL) was mixed with 2.5 mL of 0.2 M sodium phosphate buffer in a 15-mL falcon tube. A 2.5 mL of 1% potassium ferricyanide solution was then added to the tube and the tube was vortexed to mix the contents thoroughly. The mixture was then incubated at 50ºC for 20 min. A 2.5 mL of 10% trichloroaceticacid (TCA) was added after incubation and the solution was then centrifuged at 402×g for 10 min. A 2.5 mL of the upper layer was removed and 2.5 mL of deionized water was added to it, followed by 0.5 mL of 0.1% ferric chloride solution which was added at the very end. An observable color change was seen on the addition of ferric chloride. The absorbance was then measured at 400 nm. The readings for the samples were compared with those obtained for the standard (ascorbic acid 1 mg/mL) and the results reported.

Results and Discussion

Antimicrobial study Color is an important characteristic of food. Nature is rich in color and the majority of plant pigments are not widely exploited for the coloring of food. In the present study 8 food dyes were screened for their antimicrobial activity against selected bacteria and fungal organisms reported in Table 4 and 5. MTCC culture of 10 bacteria (5 pathogenic and 5 non-pathogenic) were used, of which 6 were Gram-positive and 4 Gram-negative as mentioned in Table 3A. Out of 5 fungal organism chosen, 4 were pathogenic and 1 was non-pathogenic as represented in Table 3B. All the plant dyes showed antimicrobial activity against at least 2 of the types of microorganisms tested.

The effect of concentration of dye on antimicrobial activity was studied further and results are summarized. The zone of inhibition (diameter) was recorded in each case. It was observed that increase in dye concentration leads to increased inhibition reflected by enhancement in diameter. It may be concluded that the dyes are highly effective antimicrobial agents as the minimum inhibitory concentration (MIC) for most of these lies in region of 50-500 μg. It was also observed that with increasing concentrations of dye, the zone of inhibition increased almost linearly.

Our data report that on overall, red dye showed the best antibacterial activity. The 3 red dyes tested in the study are *R. tinctorum, B. orellana,* and *P. granatum*. It works against all bacterial organisms. It has been reported by the earlier studies that these plants known to have the medicinal properties (14-16). For instance, bixin the colored compound of *B. orellana* typically serve to protect plants as well as some bacteria and fungi from photo-oxidative damage (23). Similarly *P. granatus* also has antimicrobial property owing to the huge amount of tannin present in it (9,26). *C. auriculata* and *T. erecta* yields yellow dyes from its flowers and these dyes are considered to be the next antibacterial potent followed by the red dyes.

Dye obtained from *A. catechu* and *T. chebula* is not

Table 4. Effect of food dyes against selected bacterial organisms

Plant	*Bacillus subtilis*	*Staphylococcus aureus*	*Vibrio fischeri*	*Yersinia enterocolitica*	*Proteus vulgaris*	*Lactobacillus* sp.	*Lactococcus* sp.	*Pediococcus pantosaceus*	*Staphylococcus* sp.	*Pseudomonas* sp.
Acacia catechu (L.f.) Willd	ND	ND	7.3±0.07	5.8±0.13	ND	ND	ND	ND	ND	ND
Bixa orellana L.	34.4±0.8	12.8±0.9	19.7±1.5	24.3±1.7	24.7±0.6	10.7±0.6	30.8±0.3	47.3±1.2	12±0.3	17.7±1.5
Cassia auriculata L.	13.7±0.5	32.9±2.1	31.8±0.7	24.8±0.4	6.8±0.8	34.9±1.7	13.3±0.3	ND	29.7±0.5	ND
Embillica officinalis Gaertn.	ND	ND	18±0.3	9.8±0.7	7±1.34	8.3±1.3	ND	ND	18.3±0.7	ND
Punica granatum L.	33.1±0.1	19.6±0.1	19.8±2.1	ND	33.8±0.8	18.7±0.4	11.7±0.6	26.9±0.7	26.8±0.3	19.4±1.1
Rubia tinctorum L.	21.8±0.4	6.8±0.7	20.6±0.2	34.6±1.7	11.6±0.3	24.3±0.1	21.1±1.5	22.4±0.6	42.7±1.1	13.4±0.4
Tagetes erecta L.	ND	21.1±0.6	20.4±0.1	17.6±1.1	ND	ND	23±1.9	ND	7.8±1.4	ND
Terminalia chebula Retz.	10.8±1.2	8±2.3	32.6±0.5	ND	23.2±0.2	11.7±0.2	ND	9.8±1.6	24.6±0.6	ND

Table 5. Effect of Food dyes against selected fungal organisms

Plant	*Candida albicans*	*Candida famata*	*Rodotorula* sp.	*Aspergillus* sp.	*Neurospora crassa*
Acacia catechu (L.f.) Willd	7±0.05	ND	ND	26.8±0.11	ND
Bixa orellana L.	21±0.12	ND	7.9±0.27	16.3±0.07	34.9±0.02
Cassia auriculata L.	24.2±0.7	20.8±0.37	ND	17.5±0.21	33.7±0.34
Embillica officinalis Gaertn.	ND	7.3±0.99	8±1.8	24.4±0.24	ND
Punica granatum L.	9±0.32	31.2±0.52	9.1±0.76	24.9±0.61	18.7±0.76
Rubia tinctorum L.	8.3±0.77	34.4±0.09	23.3±0.18	24±1.3	ND
Tagetes erecta L.	6.7±1.2	ND	ND	19.9±0.07	ND
Terminalia chebula Retz.	ND	32.2±0.54	17.9±0.67	18.6±0.18	ND

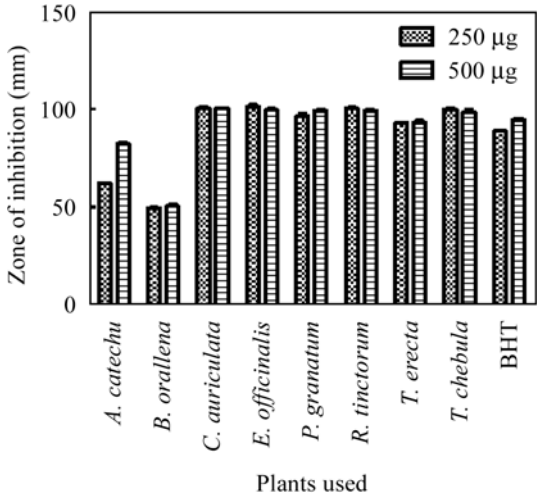

Fig. 1. Antioxidant activity by DPPH assay (inhibition at 250 and 500 µg concentration).

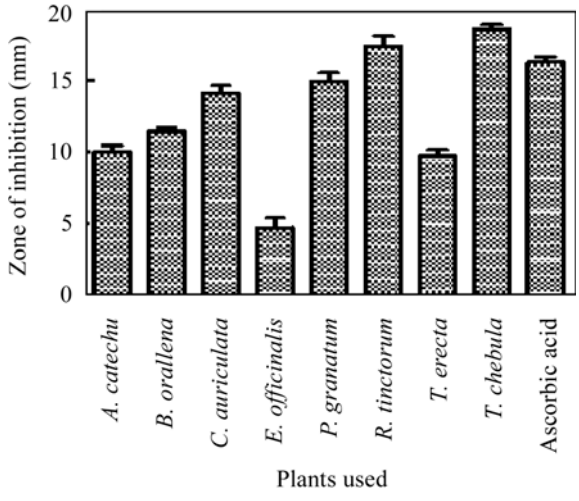

Fig 2. Results of β-carotene bleaching assay.

sufficiently effective against the tested organisms. Although both the dyes are tannin based, their anti-microbial activity differs greatly. This is an interesting finding and requires more in-depth investigation into the effect of dye structure on antimicrobial property.

In the case of antifungal study, we observed that *C. auriculata* showed best results against tested fungal organisms depicted in Table 5. *P. granatum* and *R. tinctorium* dyes have potent to good antifungal activity just as antibacterial activity. It is interesting to note that all tested dyes known to inhibit the growth of *Aspergillus* sp.

All the plants exhibited different kinds of secondary metabolites. It has been proposed that the mechanism of the antimicrobial effects involves the inhibition of various cellular processes, followed by an increase in plasma membrane permeability and finally ion leakage from the cells. Plants are rich in a wide variety of compounds, which have been found *in vitro* to have antimicrobial properties (17).

Table 6. Results of reducing power assay

Organism	Absorbance at 700 nm
Acacia catechu	0.878
Bixa orallena	0.380
Cassia auriculata	1.395
Embillica officinalis	1.980
Punica granatum	1.408
Rubia tinctorum	1.800
Tagetes erecta	1.369
Terminalia chebula	0.733
Ascorbic acid	1.872

Antioxidant assay Since dyes showed good antimicrobial activity against selected microbes, it was thought worthwhile to study their antioxidant activity. Three different methods of antioxidant assay were followed in the present study. In DPPH method, the 5 different concentrations (10, 50, 100, 250, and 500 µg) of 15 natural dyes were studied with BHT as a standard. The value of % inhibition of different concentration of each dye was analyzed and EC_{50} was calculated. Figure 1 shows the results of DPPH assay. It was observed that *T. chebula* known to have good antioxidant property. Amla dye (*E. officinalis*), *P. granatum,* and *R. tinctorum* also showed good result which has been represented in Fig. 1. The other assay, linoleic acid- β-carotene method, ascorbic acid was used as a standard. The diameter of the zones in the plates was compared to know the antioxidant activity of the dyes and results were in given in Fig. 2. In this method also *T. chebula* showed promising antioxidant activity. It is interesting to note that the linoleic acid is comparable to DPPH method as *R. tinctorum* and *P. granatum* also showed good antioxidant property. Apart from these 2 methods, the reducing power of the different natural dyes chosen was compared using ferricyanide reducing antioxidant method and ascorbic acid was used as the standard. In this method, Amla dyes showed great activity. Table 6 indicates the activity of 8 different natural dyes. *Rubia* as well as *Punica* dyes were promising in their antioxdant activities.

In all the 3 methods, one can observe that red color producing dye known to have greater antioxidant activity. These red colors are nothing but carotenoid pigments. There is convincing scientific evidence in support of the association between carotenoids and antioxidant property (18). Different carotenoids are derived essentially by modifications in the base structure by cyclization of the end groups and by introduction of oxygen functions giving them their characteristic colors and antioxidant properties. The most important biological function of carotenoids is as antioxidants owing to their potential to inactivate singlet oxygen and to quench carboxy radicals (19).

In the ever increasing world of pollution and complexities,

microbial attack is a regular feature. The various diseases caused due to the pathogenic forms of microorganisms indeed are a cause of serious concern. Moreover, regular and frequent administration of commercially available antibiotics has their own anomalies in the form of side effects and contraindications (20). Therefore, it is always a safer option to look for alternatives which can be naturally supplemented to the body through the course of food intake. Talking in like terms, the study undertaken clearly shows that the dyes under investigation are endowed with anti microbial property. Thus it invariably shall be an added advantage if the food itself is supplemented with compounds exhibiting antibiotic properties. Therefore apart from providing an aesthetic sense to the food by adding colors, these dyes can definitely prove to be an antibiotic supplement (as inhibitions have been shows against organisms like *Staphylococcus* sp., *Vibrio* sp., *Yersinia* sp., several pathogenic fungal strains, etc).

Apart from this, the other major concern which is constantly agitating the world of pharmacy is the rise in oxidative damage related disorders. The several degenerative diseases related to aging, inflammation, cardiovascular and neuronal systems which are attributed to a certain extent to the accumulation of reactive oxygen species (ROS) are believed to be curbed with the usage of antioxidants (21). Also, the recent trends in health care recommend ample use of antioxidants as a regular feature in our diet. This fact can be further reinforced with our study where we have depicted the antioxidant property of the food dyes. The various assays viz. FRAP, DPPH, and β-carotene have further substantiated the likeability of the food dyes to be applied for culinary purpose. Therefore the addition of these dyes in food not only enhances the value addition by making the food more presentable but also shall address the issue of food supplementation with substances that are good antibiotics and antioxidants, subsequently proving to be health benefactors. Moreover, each of the dyes has been conferred safe by the Food and Drug Administration (FDA) so their use in eatables is abundantly secure. In conclusion, this study ably suggests the potential usage of these reported 8 food dyes to be commercialized as a regular appearance in the food sector. Also, further research should be continued to check for the other health guarding facets of these food dyes so that their constant constructive exploration can act as a double edged sword of providing both the facilities- one of increasing the face value of the food item and second, enriching the same with health benefits through supplementation.

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