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ANTIOXIDANT AND ANTIMICROBIAL POTENTIALS OF SOME PLANT-BASED NATURAL DYES

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ABSTRACT

Natural dyes (NDs) are colourants originated from natural sources such as plants, animals, minerals and microbes. Survey of dye-producing plants in India had been done but information on the antioxidant screening of the NDs and evaluation of their antimicrobial properties remain scanty. This study aimed at evaluating antioxidant compounds in selected plant-based dyes and their antimicrobial potentials. Fifteen (15) plants were collected in Tarsadi and Bardoli, Gujarat, India. Dye was extracted from the plants using aqueous extraction method. Flavonoids, Phenolic compounds and hydroxyl radical scavenging activity of each of the NDs were determined. Antimicrobial potential of the samples was tested against *Escherichia coli, Bacillus megaterium, Proteus vulgaris* and *Enterobacter aerogenes* using agar well diffusion method. *Beta vulgaris* had highest amount of total flavonoids (1.80mg/g) and the least was found in *Daucus caurota*(0.10mg/g). *Ixora coccinea* and *Crinum augustum* had total phenolic compounds of 1.78mg/g and 1.75mg/g respectively. *Bougainvillea glabra* displayed highest hydroxyl radical scavenging activity. It is evidenced from this study that NDs from *H. rosa-sinensis* (pink), *Bougainvillea glabra, Citrus limon*and *Fragaria ananassa* were more potent against three tested bacteria than other samples. These dyes are good and recommended for textile industries to make cloths more hygienic, un harmful and pleasant. The antioxidants in NDs will benefit food and pharmaceutical industries.

Keywords: Antimicrobial, Antioxidant, Natural dyes, Textile

INTRODUCTION

Dyes (colourants) are organic compounds that give colour to substances such as paper, textile, drug, cosmetics and plastic materials. They possess colour due to their ability to absorb light in the visible spectrum, presence of colour-bearing group and exhibit resonance of electrons **[1]** Unlike paints, dyes are absorbed by the materials because the size of their molecules is smaller than the size of the pores in the materials. These colourants can be natural or synthetic. Synthetic dyes are widely used in manufacturing industries such as textile processing industries which are the major consumers and they are classified based on their chemical constitution or application **[2]**

Natural dyes (NDs) are colourants originated from natural sources such as plants, animals and minerals, although plants are the major source **[3]**. NDs are environmentally friendly as they are biodegradable and renewable. They also have no effects on skin which may even confer certain benefits to the users **[3, 4]**. These plants pigments can be used to supplant synthetic dyes due to its health benefits **[5]**. NDs are extracted from various parts of plants ranging from leaves, stems, roots, barks, fruits, flowers, wood shaving to husks. Wanyama *et al*. (2014) **[4]** extracted dyes from the bark of *Albizia coriaria, Morinda lucida, Vitellaria paradoxa* and *Syzygium cordatum*. These plants pigments can be used to supplant synthetic dyes due to its health benefits [**5**]. NDs are extracted from various parts of plants ranging from leaves, stems, roots, barks, fruits, flowers, wood shaving to husks. Wanyama *et al*. (2014) **[4]** extracted dyes from the bark of *Albiziacoriaria, Morinda lucida, Vitellaria paradoxa* and *Syzygium cordatum* **[4]**. The floral parts of *Clitoria ternatea , Ixora coccinea, Tagata erecta, Impatiens balsamina, Peltophorum pterocarpum, Lawsomia inermis, Rosa rubiginosa* and the leaves of *Croton versicolor* and fruits of *Capsicum annum* and tubers of *Beta vulgaris* were as well used for the NDs extraction **[6]**. Ixora dye widely used in textile industries **[7]**. Textile industries play an important role in the economy especially in Asian countries. It accounts for the largest consumption of dyestuff at 80% in India **[6]**.

Phytochemical screening helps identify bioactive substances in plant. Some of these secondary metabolites are flavonoids, tannins, saponins, glycosides, alkaloids and antioxidants **[8, 9 & 10]**. NDs have bioactive compounds with antimicrobial, analgesic, and anti-inflammatory properties **[10]**. Survey of dye-producing plants in India had been done **[11]** but information on the antioxidant screening of the NDs and evaluation of their antimicrobial properties remain scanty. Besides, antimicrobial evaluation of the NDs from plants become necessary especially in textile industries to avoid microbial infection, reduce bad odour and maintain good hygienic condition of the textile **[8]**. This study aimed at screening the antioxidant compounds in selected plant-based natural dyes and their antimicrobial potentials.

MATERIALS AND METHODS

Collection of Materials

The plant samples were collected in around Bardoli and Tarsadi in Gujarat State (Western India). The names and parts of the plants used were given in **Table 1**.

Extraction of Dye from Plant Parts

Dye extraction was carried out using aqueous extraction method **[6]**. The cleaned samples (50g) were crushed, dissolved in deionized water (500 ml) and then boiled for 2 hours in a hot water bath for quick extraction. At the end of 2 hours, the total color was extracted. The solution was then double filtered and used to carry out this study.

Antioxidant Determination

Total flavonoid content (TFC)

The total flavonoid content of a sample was determined by the aluminum chloride colorimetric method **[12]**. Briefly, 0.5 mL of a dried extract ethanolic solution (1:10, w/v) was mixed with 0.5 mL methanol, 50 mL of 10% Aluminum chloride, 50 mL of 1.0 mol L^{-1} potassium acetate and 1.4 mL distilled water. The mixture was left to stand for 30 min at room temperature (25^{o}C), thoroughly mixed using a vortex mixer and then the absorbance was read at 510 nm using a UV-spectrophotometer. A calibration curve was prepared from different concentrations of quercetin (R2 = 0.996). The TFC was calculated from the calibration curve.

Table 1: List of the Plants used with their Voucher Numbers

S/No	Common Name	Botanical Name	Family	Part of the Plant Used	Voucher Number
1	Pink Rose	*Rosa rubiginosaL*	Rosaceae	Flower	CGBIBT/Rr/1-1
2	White Rose	*Rosa rubiginosaL*	Rosaceae	Flower	CGBIBT/Rr/1-2
3	Red Hibiscus	*Hibiscus* rosa-sinensis L	Malvaceae	Flower	CGBIBT/HRs/1-1
4	Pink Hibiscus	*Hibiscus* rosa-sinensis L	Malvaceae	Flower	CGBIBT/HRs/1-2
5	Baramasi	*Catharanthus roseus* (L) G.Don	Apocynaceae	Flower	CGBIBT/Cr/1-1
6	CrinumLily	*Crinum augustumL.*	Amaryllidaceae	Flower	CGBIBT/Ca/1-1
7	Marigold	*TageteserectaL.*	Asteraceae	Flower	CGBIBT/Te/1-1
8	Jungle Flame	*Ixora coccinea* L.	Rubiaceae	Flower	CGBIBT/Ir/1-1
9	*Bougainvillea*	*Bougainvillea glabra* (Juss.)Comm.	Nyctaginaceae	Flower	CGBIBT/Bg/1-1
10	Asian pigeonwings	*Clitoriaternatea*L.	Fabaceae	Flower	CGBIBT/Ct/1-1
11	Carrot	*Daucus carota* L.	Apiaceae	Root tuber	CGBIBT/Dc/1-1
12	Beet	*Beta vulgaris* L.	Amaranthaceae	Root tuber	CGBIBT/Bv/1-1
13	Straw berry	*Fragaria ananassaDuch.*	Rosaceae	Fruit	CGBIBT/Fa/1-1
14	Mustard	*Brassica nigra L.*	Brassicaseae	Flower	CGBIBT/Bn/1-1
15	Lemon	*Citrus limon* (L) Osbeck.	Rutaceae	Fruit	CGBIBT/Cl/1-1

Total Phenolic Contents (TPC)

The total phenolics of NDs from the selected plants were determined by the Folin-Ciocalteu's reagent method **[13]**. An aliquot (1.0 mL) of an aqueous solution of each sample was added to 2.0 mL of 2% sodium carbonate (Na_2CO_3). After 5 min, 5.0 mL of 0.2 mol L^{-1} Folin-Ciocalteu's reagent was added to the mixture and thoroughly agitated for 10 min. Then, 4.0 mL of (75 g L^{-1}) sodium carbonate was added to the mixture and allowed to stand in the dark for 2 h at 20°C. The absorption was measured at 760 nm using a UV-spectrophotometer (T60, Beijing Purkinje General Instrument Co., Ltd, China). Standard curve was prepared from different concentrations of Gallic acid dissolved in 75% ethanol (R2 = 0.994). The TPC was calculated from the calibration curve, and the results were expressed as mg of Gallic acid equivalent per g dry weight.

Hydroxyl Radical Scavenging Assay

Briefly, 1 mL of varying concentrations (10 – 100%) of the culture filtrate was added to 600 µL of FeSO4 (8 mM), 500 µL of H_2O_2 (20 mM) and 2 mL of salicylic acid (3 mM) in a test tube. Following a 10 min incubation period at 37°C, distilled water (2.9 mL) was added and the resulting mixtures were shaken and allowed to settle for 15 min. The absorbance was subsequently read at 510 nm and percentage OH radical scavenging capacity of the filtrate was estimated as per the expression:

$$\textbf{Percent hydroxyl radical scavenged}\ (\%) = \frac{(\textbf{Acontrol} - (\textbf{Asample} - \textbf{ANDs})}{\textbf{Acontrol}} \times \textbf{100}$$

Where, $A_{control}$, A_{sample} and ANDs represent the absorbance of the mixture without NDs, mixture with the NDs and that of the NDs alone, respectively.

Antibacterial Assays

The agar-well diffusion method was used for monitoring the antibacterial potentiality of NDs against *Escherichia coli, Bacillus megaterium, Proteus vulgaris* and *Enterobacter aerogenes* with gentamicin 30 mg/mL as the standard **[14]**. Antibacterial potential was determined by measuring the diameter values of zones of inhibition.

RESULTS AND DISCUSSION

Antioxidant Activities

All NDs from plant samples had flavonoids but with different concentrations **(Figure 1)**. Flavonoids are known for their antioxidant properties that possess anti-cancerous, antimicrobial, anti-allergic and anti-inflammatory activity **[8, 15]**. *Beta vulgaris* had highest amount of TFC (1.80mg/g) and the least was found in *Daucus caurota* (0.10mg/g). Flavonoids are polyphenolic compounds with strong antioxidant features. Presence of this free radical scavengers in *B. vulgaris* may be responsible for its ability to be used as an adjuvant in amelioration oxidative and inflammation conditions such as hypertension, atherosclerosis, type 2 diabetes and dementia **[16]**. Beetroot extracts has currently gained much attention in food industries as colourant due to its flavonoid contents with anti-inflammatory properties **[17]**. From the result of this study, *Bougainvillea glabra* had power for oxidative stress and related diseases management**[15]**The quantity of flavonoids in *Rosa rubiginosa* (Pink) (1.35mg/g) is insignificantly ($p < 0.05$) higher than that of *Rosa rubiginosa*(White) (1.11mg/g). the amount of flavonoids in each of the two NDs is less than 2.46 mg/g as reported **[18]**. The difference may be due to regions of sample collections. It has been previously reported that among all *Rose* species, *R. rubiginosa* has highest amount of flavonoids and this quality makes the species a good candidate in natural dye production **[19]**. Generally, flavonoid compound gives an appealing colour to food and other industrial products which is one of vital characteristics for the ultimate initial acceptance of a product by the consumers. Anthocyanin (a flavonoid) imparts taste in addition to potential influence of colour, antioxidant power and protection of vitamins and enzymes **[20]**. *Brassica nigra* had 1.33mg/g of flavonoids. This has been corroborated that mustard has preservative and antioxidant properties in addition to providing flavour and used as dye **[21]**.

Phenolic compounds are a group of plant-based biologically active compounds with a strong antioxidant properties and have anti-inflammatory and anticancer potentials **[22]**. These compounds are also antimicrobial **[23]**. *Ixora coccinea* and *Crinum augustum* had phenolic quantities of 1.78mg/g and 1.75mg/g respectively (Figure 2).*I. coccinea* flower is very rich in phenolic compounds which was estimated qualitatively using Thin Layer Chromatography **[24]**. This may account for the good quality of *I. coccinea* dyed fabric with pomegranate rind as natural mordant that ensure a very good rubbing and light fastnesses **[24]**. *Crinums* species are widely used in traditional folk medicine due to their excellent source of bioactive principles such as alkaloids and phenolic compounds **[25]**. The aqueous dye extracted from *Targetes erecta* had 1.59mg/g of phenolic compounds which is lower than value (48.2mg/g)reported by **[26]**. This disparity may be as a result of extractant used. Previous work revealed that a similar species, *T. patula* flower extract contains flavonoid and phenolic acid which are responsible for its cytotoxic and antioxidant properties **[27]**. This finding can be likened to this present study because *T. erecta* also contained both bioactive principles. [**28]** reported that *T.*

erecta flower extract have a significant antioxidant activity close to the standard as a result of this quality, it can be used as an alternative to synthetic antioxidants.

Rosa rubiginosa (White) had highest potential to scavenge hydroxyl radicals. The antioxidant activity of rose ship extract is expected due to the presence of ascorbic acid and phenolic compounds in its extracts. Hydroxyl radical reacts will polyunsaturated fatty acid moieties of cell membrane phospholipids and damage the cells. This assay is important to check the scavenging activity of free OH^- radicals. All the NDs used in this study have certain levels of OH^- radical scavenging activity **(Figure 3)**. The HRS of NDs from *B. glabra* flower was 1.00%. This is an indication that the plant part has hydroxyl scavenging ability and this was confirmed **[29]**.

Antimicrobial Assay

Among all the tested NDs, *H. rosa-sinensis* (pink) had highest inhibition zone against *E. coli* (31.33mm) but it was insignificantly different from what was observed in *Bougainvillea glabra(30.67mm), Citrus limon* (29.33mm) and *Fragaria ananassa* (28.00mm). The zone of inhibition of all the NDs against *E.coli* was higher than the control except *Rosa rubiginosa (White), Hibiscus rosa sinensis (Red), Cantharanthus roseus, Ixora coccinea, Beta vulgaris* and *Brassica nigra* **(Table 2)**. The inhibition zone recorded in the control (28.33mm) against *Bacillus megaterium* was significantly higher than most of those in NDs. However, the antimicrobial potentials of *Hibiscus rosa-sinensis* (Pink)*, Bougainvillea glabra, Clitoria ternatea* and *Citrus limon* were more pronounced than control. *Fragaria ananassa, Citrus limon*, and *Bougainvillea glabra displayed maximum antimicrobial ability against Proteus vulgaris.* For the *Enterobacter aerogenes, B. glabra* inhibited the growth of the bacterium *most (31.67mm), follow by C.limon* (29.67mm) and *Fragaria ananassa* (29.00mm).

It is evidenced from this study that NDs from *H. rosa-sinensis* (pink), *B. glabra, C.limon*and *F. ananassa* are more potent against the three tested bacteria. It was reported that *H. rosa-sinensis* (pink) has anthelmintic, hypotensive, anti-inflammatory, astringent, purgative and antimicrobial properties **[8]**. The inhibition of bacterial growth by the extract of the flower could be due to the some bioactive compounds that act alone or in combination to perform the inhibitory action **[30]**. The antimicrobial potential of *B. glabra* against the growth of bacteria was also corroborated **[15]**. *B. glabra* has been used in Mexican traditional folk medicine for the treatment of respiratory diseases such as cough, asthma and bronchitis **[31]**. Besides antimicrobial activity of *C. limon* extract, it used for weight loss, skin care, piles, urinary disorders, treatment of scurvy and peptic ulcer **[32]**. Ability of *F.ananassa* to inhibit the bacterial growth may be the product of synergic activities of phenolic and anthocyanin compounds that are present in the extract of the fruits **[33]**.

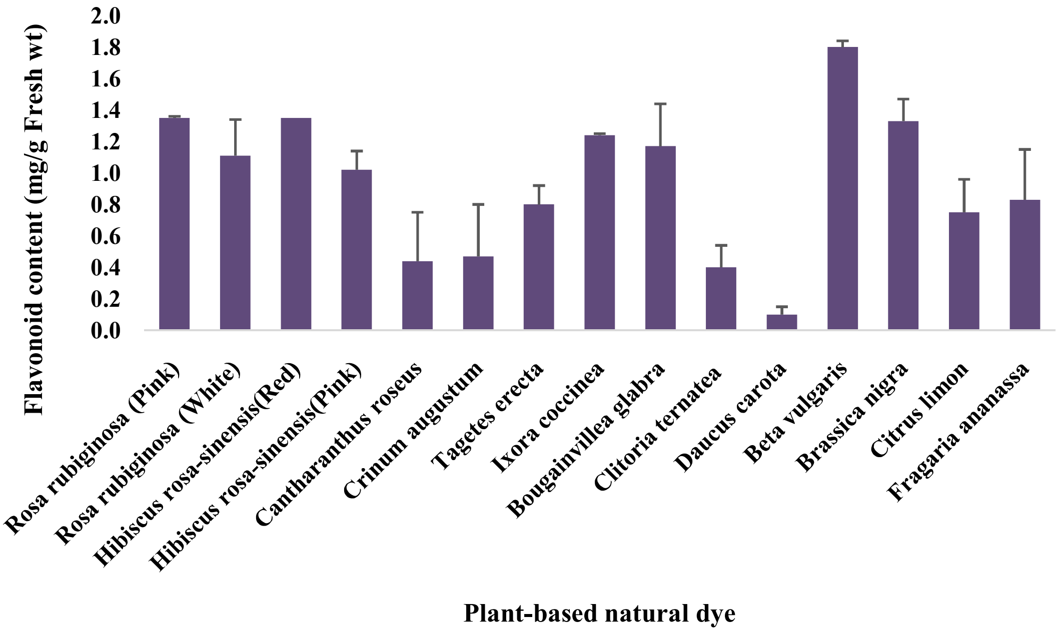

Figure 1: Concentration of Flavonoids in Plant-Based Natural Dyes

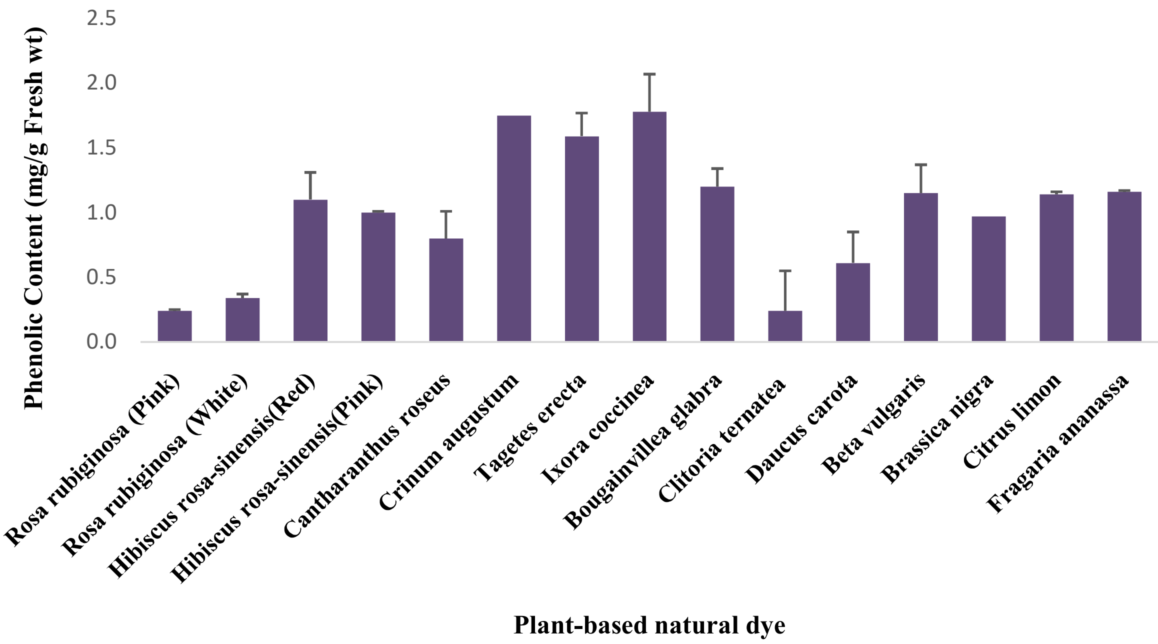

Figure 2: Concentration of Phenolic content in Plant-Based Natural Dyes

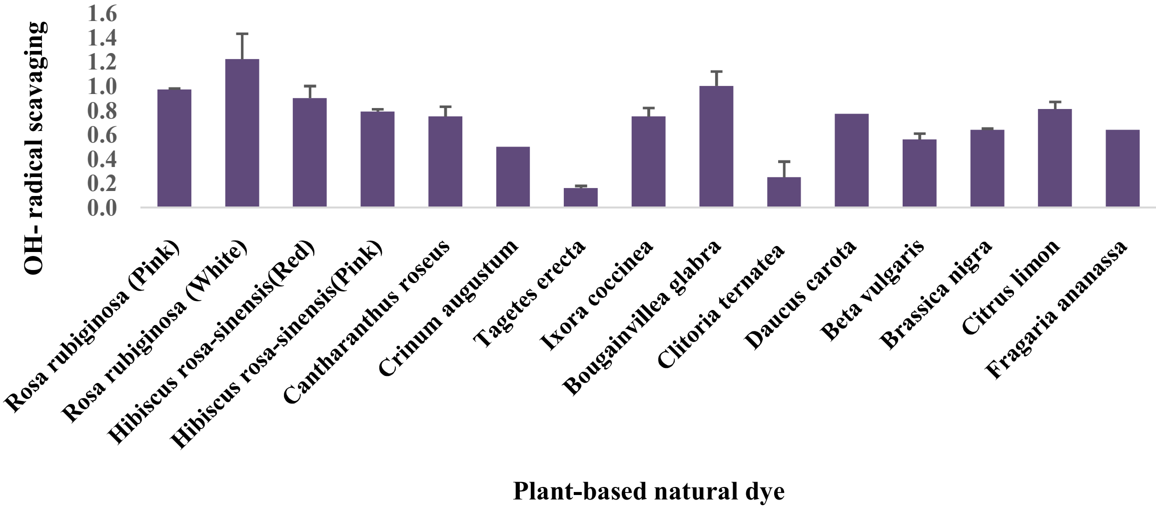

Figure 3: OH- Radical Scavenging activity in Plant-Based Natural Dye

Table 2: Antibacterial potentials of Plant-based Dyes

S/No	Name of the Plant	Diameter of Inhibition Zone (mm)			
		Escherichia coli	*Bacillus megaterium*	*Proteus vulgaris*	*Enterobacter aerogenes*
1	Control	18.33 ± 0.33^{cdef}	28.33 ± 1.76^{c}	22.00 ± 1.00^{cd}	22.00 ± 0.57^{c}
2	*Rosa rubiginosa (Pink)*	14.00 ± 2.31^{g}	27.33 ± 0.88^{c}	20.33 ± 2.91^{de}	14.67 ± 0.33^{d}
3	*Rosa rubiginosa (White)*	19.33 ± 0.33^{cde}	14.00 ± 1.53^{g}	14.33 ± 0.88^{fg}	11.00 ± 2.65^{de}
4	*Hibiscus rosa-sinensis(Red)*	17.67 ± 0.33^{cdefg}	22.67 ± 1.45^{de}	16.33 ± 1.20^{efg}	09.67 ± 1.76^{e}
5	*Hibiscus rosa-sinensis(Pink)*	31.33 ± 1.45^{a}	35.67 ± 0.33^{b}	22.00 ± 1.73^{cd}	24.33 ± 0.33^{c}
6	*Cantharanthus roseus*	16.00 ± 1.00^{efg}	29.00 ± 0.58^{c}	14.33 ± 0.88^{fg}	14.33 ± 0.33^{de}
7	*Crinum augustum*	20.67 ± 2.19^{cd}	22.33 ± 1.67^{de}	14.67 ± 0.67^{fg}	13.33 ± 1.86^{de}
8	*Tageteserecta*	26.33 ± 0.58^{b}	19.67 ± 1.20^{ef}	11.67 ± 0.88^{g}	24.67 ± 2.03^{bc}
9	*Ixora coccinea*	14.67 ± 0.33^{fg}	15.33 ± 0.33^{g}	$19.00\pm1.00d^{ef}$	13.00 ± 0.00^{de}
10	*Bougainvillea glabra*	30.67 ± 0.88^{a}	36.67 ± 2.03^{b}	35.67 ± 2.03^{a}	31.67 ± 1.76^{a}
11	*Clitoriaternatea*	26.33 ± 1.20^{b}	35.33 ± 0.33^{b}	29.33 ± 2.60^{b}	20.67 ± 2.40^{c}
12	*Daucus carota*	21.33 ± 1.20^{c}	21.33 ± 1.86^{de}	26.00 ± 1.00^{bc}	10.00 ± 1.16^{de}
13	*Beta vulgaris*	17.00 ± 1.00^{defg}	23.67 ± 0.82^{d}	12.00 ± 0.58^{g}	11.33 ± 1.20^{de}
14	*Brassica nigra*	13.67 ± 0.88^{g}	16.67 ± 0.33^{fg}	14.67 ± 1.87^{fg}	25.00 ± 0.00^{bc}
15	*Citrus limon*	29.33 ± 1.20^{ab}	40.33 ± 1.20^{b}	34.00 ± 0.00^{a}	29.67 ± 0.88^{a}
16	*Fragaria ananassa*	28.00 ± 2.30^{ab}	26.50 ± 1.20^{a}	35.33 ± 0.67^{a}	29.00 ± 2.08^{ab}

Mean ± SE (n = 3). Means with different superscript letter(s) in the same column significantly different (P<0.05).

CONCLUSION

All the NDs from plant materials used in this present study have considerable amount of antioxidant compounds which are very essential in biological system. Beta vulgaris dye is very rich in flavonoid compounds and phenolic compounds are abundantly present in *Ixora coccinea. H. rosa-sinensis* (pink), *Bougainvillea glabra, Citrus limon*and *Fragaria ananassa* are excellently potent against the tested bacteria. These NDs can be used to proffer solution to resistance menace of bacteria against the existing synthetic antibiotic drugs. These dyes are good in textile industries to make clothes more hygienic, unharmful and pleasant. The colourants are also alternative to supplant synthetic food additives that cause health hazards because of their antioxidant and antimicrobial properties.

This study provides scientific evidence on the free radical scavenging ability and antimicrobial potential of natural dyes from plant materials. Further studies are suggested to carryout *in vivo* evaluations of these plants to complete preclinical trial in a drug discovery system.

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