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High selective purification of flavonoids from natural plants based on polymeric adsorbent with hydrogen-bonding interaction

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ABSTRACT

The efficient purification method of high purity flavonoids from natural plants was reported. A series of polymeric adsorbents with novel structure were synthesized based on the copolymerization of methyl acrylate (MA) and ethylene glycol dimethacrylate (EDGMA). Functional groups, such as ester, amino or amide group, were introduced into the adsorbent matrix, respectively, to produce the hydrogen-bonding interaction and enhance the adsorption selectivity towards flavone compounds. The influences of matrix structure and functional groups of synthesized adsorbents on the adsorption selectivity were investigated. The resins were applied to purify flavonoids in natural plants. It was illuminated that the adsorbent No. 3B with 15% EGDMA content and amide groups performed optimal selectivity to flavone compounds in *Scutellaria barbata* D.Don, from which the purity of flavonoids in extracts was obtained more than 50%, obviously higher than that from commercial adsorbents. The result of adsorption thermodynamics experiment showed that the isosteric adsorption enthalpy of No. 3B was in the range of 25–30 kJ/mol, which testified that the adsorption mechanism was related to hydrogen-bonding interaction. The method showed its universality via good effects on the purification of total flavonoids from *Ginkgo biloba* L., *Radix puerariae* and *Hypericum perforatum* L.

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1. Introduction

Flavonoids, abundant in natural plants, have been applied in health products, cosmetics and medicines due to their biological and physiological activities. According to modern pharmacology research, flavonoids have anti-inflammatory, antitumor, antioxidant and free-radical scavenging properties [1,2], and have been widely used in the research and development of the natural medicine as well as in clinical application. An extensive prospect for further applications of flavone compounds is expected [3,4].

Of all the methods for extraction and purification of natural flavone compounds, solvent extraction turned out to be a traditional and mature technique [5,6]. But in recent years, with the increasing awareness of problems in environment pollution and residual solvents in solvent extraction process, it was of greater significance to develop a simpler and more environment-friendly technique with high efficiency, low toxicity, and high selectivity. Macroporous resin has been developed into a novel technique for the separation of active components, such as flavonoids, alkaloid [7] and saponins [8] from natural plants. Because of its high efficiency, low pollution, and procedural simplicity, macroporous resin is also suitable for industrial application.

There had been some reports on the application of the commercial polymeric adsorbents in the enrichment process of flavonoids [9–14]. The matrix of these resins was usually of hydrophobic polystyrene or polyacrylic ester, and the absorption mechanism primarily relied on the hydrophobic force such as the van de Waals force in the aqueous solution, however, which may lead to the low adsorption selectivity [15]. Certain adsorbates, such as flavonoids, have special structures which have multi-phenolic groups and a hydrophobic skeleton, as shown in Fig. 1. The structure indicates that if introducing some special functional groups onto current resin matrix to activate the hydrogen bond interaction between adsorbents and flavonoids, higher purity flavonoids may be achieved from the complicated system of natural plants. Nevertheless, it hardly lives up to our expectation in practical applications. Only depending on macroporous resins, it is difficult to obtain high purity flavonoids extract from the natural plants directly in present researches, without the support of some additional methods [16–18]. One specific commercial resin can obtain preferable purity power from a certain plant, but cannot be applied to all plants. For example, ADS-17 is a good adsorbent to get total flavonoids with relatively high purity from *Ginkgo biloba* L. [19], but purity even lower than polystyrene resins when

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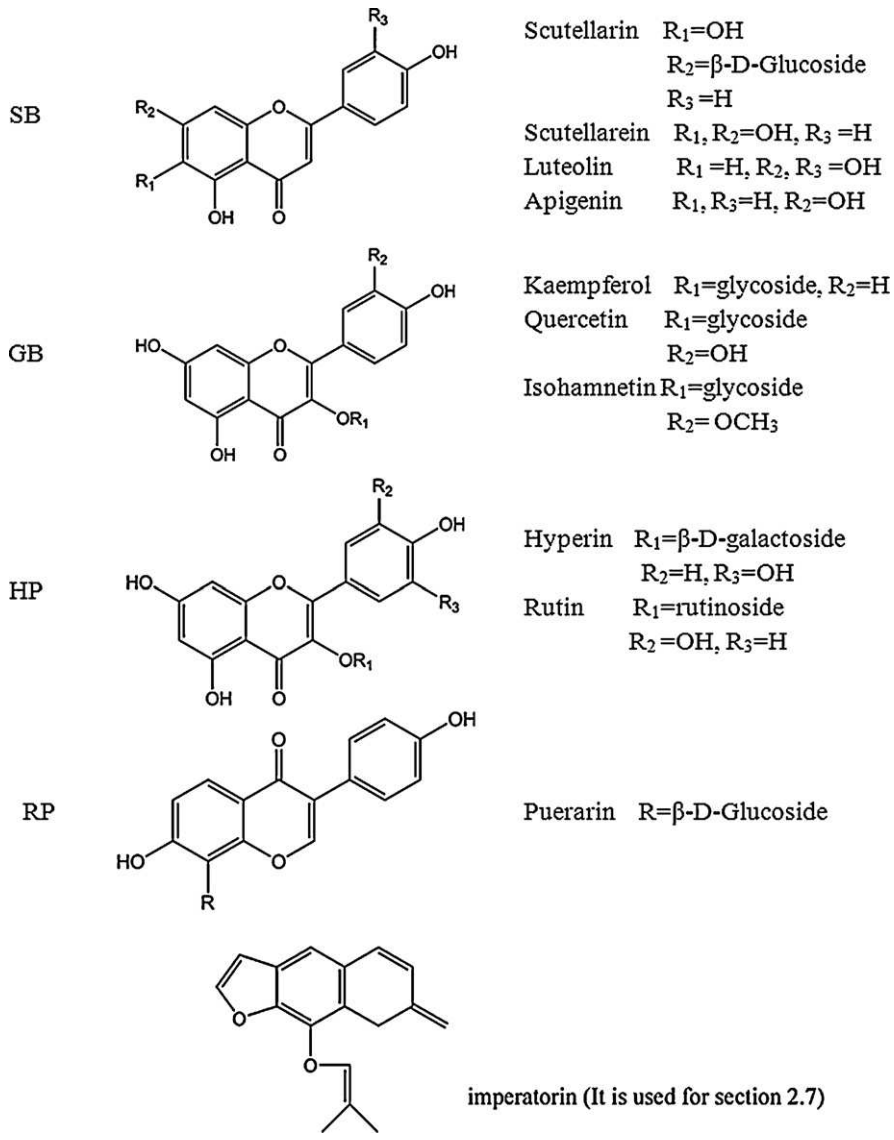

Fig. 1. Structures of flavonoids in the selected four natural plants.

applied to other plants. Essentially, only introducing hydrogen bond to the polystyrene absorbents cannot enhance the selectivity to flavonoids. So, the universality of the extraction method based on these commercial resins is quite low. In the current studies, the resins used in different plants systems for extracting flavone compounds are mainly obtained based on the screening experiment over large scope and a special resin is still lacking, because there are no general principles for the control of the resin configurations. All these problems are caused by the fact that, as Maity and Payne [20] clarified, the adsorption selectivity of hydrogen bonding is always covered by excessively strong hydrophobic interaction between solute and polystyrene matrix in aqueous solution [21]. However it has been difficult to exploit the hydrogen-bonding mechanism in aqueous environments between the adsorbate and the functional group on the adsorbent matrix, for water's own ability to form the hydrogen bonding. In this regard, Schrader's group considered that hydrophobic interaction with certain intensity is quite necessary for the hydrogen-bonding interaction between adsorbate and adsorbent, because it could assist to avoid the influence of water and drive the adsorbate to get closer to the adsorbent [22]. The hydrophobicity of adsorbents has a subtle influence in the adsorption process. Accordingly, the key strategy in resin designing is to adjust both the hydrophobic and hydrogen-bonding interaction synergistically.

In the present paper, a series of adsorption resins with novel matrix and hydrogen-bonding groups were synthesized based on the investigation of the hydrogen bonds interactions between adsorbent and adsorbate. A weak polar polyacrylate was used as the resin matrix, meanwhile amide groups were introduced. Four Natural medicinal plants, including *Scutellaria barbata* D.Don (SB) [23,24], *G. biloba* L. (GB) [25,26], *Radix puerariae* (RP) [27,28] and *Hypericum perforatum* L. (HP) [29,30], were chosen to study practical application effect. The purity of flavonoids in the final products verified that the method for regulating resin configurations is applicable to the purification of flavonoids. The relationship among hydrophobicity, functional groups and adsorption selectivity to flavonoids of the resins was investigated. The results showed that the adsorption selectivity of the selected synthesized resin was remarkable, and the purity of flavonoids in the products was much higher than those obtained from the commonly used adsorbents. Furthermore, the method established showed its universality among the four flavonoids systems.

2. Experimental

2.1. Materials

Methyl acrylate (MA, AR) and ethylene glycol dimethacrylate (EGDMA, AR) were obtained from Chemical Plant of Nankai University (Tianjin, China). 2,2′-Azobisisobutyronitrile (AIBN), acetic anhydride, ethylenediamino, heptane, butyl acetate, dimethylformamide (DMF), phosphoric acid, sodium chloride and ethanol were purchased from Tianjin Chemical Co. (Tianjin, China) and were of analytical grade.

Whole grass of *S. barbata* (SB), leaf of *G. biloba* L. (GB), *H. perforatum* L. (HP) and root of *R. puerariae* (RP) were employed after washing and drying. The structures of their main flavone compounds were shown in Fig. 1. Their standards and imperatorin were purchased from J&K Chemical Ltd. (Beijing, China). All solutions prepared for HPLC were filtered through 0.45 μm nylon membranes before use.

2.2. Pretreatment of commercial adsorbents

Macroporous adsorbent Amberlite XAD-4 was purchased from Rohm and Hass (Philadelphia, PA, USA). Macroporous adsorbents D380, ADS-17, ADS-21 and AB-8 were purchased from Nankai Hecheng S&T (Tianjin, China). The adsorbents were eluted with light petroleum (b.p. 60–90 °C) to remove the monomers and porogenic agents trapped in the pore, and then dried at 60 °C under vacuum [31]. Prior to the adsorption experiments, the adsorbents were soaked in ethanol and subsequently washed with deionized water thoroughly [32].

2.3. Synthesis of the adsorbents with functional group

2.3.1. Preparation of the beads with ester group

The initial beads were prepared using a suspension polymerization method. An organic solution composed of methyl acrylate (MA), ethylene glycol dimethacrylate (EGDMA), porogenic agent (heptane and butyl acetate, 50%, w/w) and initiator AIBN (0.5%, w/w) was mixed with the aqueous solution composed of polyvinylalcohol (PVA, 1%, w/w) and sodium chloride (5%, w/w) in a 1000 mL three-necked round-bottomed flask equipped with a mechanical stirrer, a reflux condenser and a thermometer. The round-bottomed flask was heated by a programmed heater. The mixture was stirred to give a suspension of oil beads with a suitable size on the aqueous solution (100–120 rpm), then heat at 65 °C for 4 h and keep at 85 °C for 6 h. The copolymer beads were filtered out, washed with a large amount of hot water, followed by ethanol, then packed in a Soxhlet extractor and eluted with petroleum ether.

Because the hydrophobicity of EGDMA was stronger than that of MA, the variation of the proportion between them in the synthesis process can obtain polymeric adsorbents with different hydrophobic affinities. The adsorbents were named No. 1, No. 2, No. 3, No. 4, and No. 5, corresponding to 5%, 10%, 15%, 20%, and 25% EGDMA content in weight, respectively.

2.3.2. Preparation of the beads with amino and amide group

The initial beads were air-dried, then swelled with dimethylformamide (DMF) for 8 h and mixed with excessive 1,2-ethylenediamine in a 500 mL three-necked round-bottomed flask equipped with a mechanical stirrer, a reflux condenser and a thermometer. The mixture was heated at 110 °C for 9 h and beads with amino group were synthesized through ester-exchange reaction. Then the beads were filtered out and washed with ethanol followed by deionized water, and air-dried. The beads with amino group were named No. 1A, No. 2A, No. 3A, No. 4A, and No. 5A obtained from No. 1, No. 2, No. 3, No. 4 and No. 5.

The beads with amino group were mixed with acetic anhydride in a 500 mL three-necked, round-bottomed flask in the same manner as described above. The mixture was heated at 75 °C for 8 h. Then, the beads with amide group were obtained through acylating reaction of amino group and were named No. 1B, No. 2B, No. 3B, No. 4B, and No. 5B.

2.4. Determination of the adsorbents' physical parameters

2.4.1. Determination of pore structure

Pore structure parameters of the adsorbents synthesized were measured using an automatic surface area analyzer (Autosorb-1-MP, Quantachrome Instruments, Boynton Beach, FL, USA) based on the BET nitrogen adsorption method.

2.4.2. Determination of the amount of amino group in adsorbents

The amounts of amino group in the synthesized adsorbents before and after acylating reaction were determined according to the literature [21] method. Amino group content (N, mol/g dry adsorbent) in the adsorbents was calculated from the following equation:

$$N = \frac{(50C_{HCl} - V_{NaOH}C_{NaOH})10^{-3}}{W_{wet}(1-\alpha)} \quad (1)$$

where V_{NaOH} (mL) is the volume of standard NaOH aqueous solution used in titration. C_{HCl} and C_{NaOH} (mol/L) are the concentrations of the standard HCl and NaOH aqueous solution, respectively. W_{wet} is the weight of wet adsorbent (g). α is the moisture content imbedded in the adsorbent. The determination ofα is described in Section 2.4.3.

2.4.3. Determination the moisture proportion imbedded in the adsorbent bead

The hydrated adsorbents disposed of deionized water were weighed accurately and then dried in an oven at 110 °C until constant weight. The following equation was used to calculate the moisture content imbedded in the adsorbent.

$$\alpha = \frac{W_{wet} - W_{dry}}{W_{wet}} 100\% \quad (2)$$

where α is the moisture content imbedded in the adsorbent (%), W_{wet} is the weight of the hydrated adsorbent (g), W_{dry} is the weight of the dry adsorbent (g).

2.5. HPLC analysis

All analyses were carried out by a HPLC system, which consisted of Waters 510 HPLC pump and Waters 486 UV detector. A reversed column packed with Kromasil C18 (5 μm, 4.6 mm× 200 mm) was used for chromatographic separation.

It is well known that the kinds of the flavone compounds in the natural plants should be varied although the aglycone structure is almost same. This is because the kind, number or position of the substituting group on the aglycone molecular structure maybe not same. In this article, several different flavonoids (as listed in Table 1) were selected as the objective components from the corresponding herb. These flavonoids were considered as the principal and active components in each herb according to the literature [33–36]. In addition, due to different components in each herb, their corresponding HPLC detection conditions were chosen and also listed in Table 1. The flow rate was 0.8 mL/min and the injection volume was 20 μL in all analytical processes.

Table 1
HPLC detection conditions of flavonoids content in medicinal plants.

Herbs	Flavone compounds	Mobile phase	Detection wavelength (nm)
SB	Scutellarin, scutellarein, luteolin, apigenin [33]	MeOH–H_2O–H_3PO_4 (40:60:0.3, v/v/v)	335
GB	Kaempferol [34]	MeOH–H_2O–H_3PO_4 (55:45:0.3, v/v/v)	368
RP	Puerarin [36]	MeOH–H_2O (25:75, v/v)	250
HP	Hyperin, rutin [35]	MeOH–H_2O–H_3PO_4 (30:70:0.3, v/v/v)	360

2.6. Preparation of the sample solutions

The sample solutions of crude extraction of four herbs were prepared as follows: The dried powder of herb (100 g) was extracted with 800 mL of ethanol solution, heated and refluxed at 60 °C for 2 h. The extracts after filtered were concentrated to dryness by removing the ethanol solvent under reduced pressure in the rotary evaporator at 60 °C. The residues were dissolved in 500 mL water. After filtering, the transparent solutions were sample solutions. The concentrations of all sample solutions were determined by HPLC analysis.

2.7. Static adsorption and desorption tests

Rutin was selected as a model of flavonoids, while imperatorin was a model of compound without hydrogen group and the structure was shown in Fig. 1. 0.3 g each of rutin and imperatorin was dissolved in 500 mL aqueous solution, respectively, to prepare sample solution. The static adsorption tests were carried out as follows: 4 g hydrated test adsorbent was put into 100 mL flask with a lid, 30 mL sample solution was added. The flask was then shaken (100 rpm) at a constant temperature of 25 °C until the adsorption equilibrium reached. The concentrations of rutin and imperatorin in the solution after the adsorption were analyzed. The following equation was used to quantify the adsorption capacity of the adsorbent:

$$Q_e = \frac{(C_0 - C)V_i}{W_{wet}(1-\alpha)} \quad (3)$$

where Q_e is the adsorption capacity at the adsorption equilibrium (μg/g resin); C_0 and C are the initial and equilibrium concentrations of the adsorbate in the solutions, respectively (μg/mL); V_i is the volume of the sample solution (mL); W_{wet} is the weight of the hydrated adsorbent (g); α is the moisture content imbedded in the adsorbent (%).

After adsorption process, the adsorbents were washed with deionized water and then were put into 100 mL flask with a lid, and 50 mL 80% ethanol desorption solution was added. The flask was then shaken (100 rpm) at 25 °C until the equilibrium reached. The concentrations of rutin and imperatorin in desorption solution were analyzed. The following equations were used to quantify the desorption ratio.

$$D = \frac{C_d V_d}{(C_0 - C)V_i} 100\% \quad (4)$$

where D is the desorption ratio (%), C_d is the concentration of the adsorbate in the desorption solution (mg/mL), V_d is the volume of the desorption solution (mL), C_0, C and V_i are the same as above.

2.8. Determination of the adsorption isotherms

Isotherm experiments were carried out using the method as follows: about 0.5 g of dry resin was firstly wetted and was put into flasks with lid, respectively. 50 mL of the aqueous solution of rutin with known concentration was added into each flask. The flasks were shaken for 12 h at a temperature of 290 K, 300 K, 310 K until adsorptive equilibration, respectively. The concentrations of rutin at equilibrium were determined by UV-spectrophotometer (T6, C11948 UV-VIS, Beijing Purkinje, China). The initial concentrations of the solutions were over the range of 20–55 mg/L depending on the water solubility of rutin. The adsorption capacity at adsorption equilibrium Q_e (mg/g) was calculated according to Eq. (3).

2.9. Dynamic adsorption and desorption tests

Dynamic adsorption and desorption experiments were carried out in glass columns (Ø = 15 mm, 12 mm × 500 mm) wet-packed with 40 mL of the adsorbent selected, respectively. 50 mL of sample solution flowed through the glass column at the flow rate of 20 mL/h. After adsorption, the columns were washed first with deionized water, then eluted by desorption solution, the flow rate was 20 mL/h, the concentrations of flavone compounds in the desorption solution were determined by HPLC analysis. The desorbed solution was vacuum-dried after removing solvent, and the product was obtained.

3. Results and discussion

3.1. Purification effects of selected commercial resins towards flavonoids

The commercial resins ADS-21, D380, ADS-17, AB-8 and XAD-4 were selected in order to study the relationship between structures of adsorbents and adsorption performances. Their physical properties were listed in Table 2. Purification effects of these five commercial resins towards flavonoids in the selected natural plant were studied, and the results were listed in Table 3.

As shown in Table 3, the resin AB-8 and XAD-4 were polystyrene resin, and their selectivities to flavonoids were poor for their hydrophobic adsorption mechanisms. A large amount of hydrophobic impurities were adsorbed simultaneously with flavone compounds. Thus, the purities of flavone compounds in the products obtained from them were quite low.

Generally speaking, the purification effect of D380 and ADS-21 was superior to that of other resins without hydrogen groups. It was because there were hydrogen bonds interaction between the

Table 2
Physical properties and functional group of commercial resins.

Adsorbent	Functional group	Matrix	Surface area (m^2/g)	Particle diameter (mm)
XAD-4	–	P(St-DVB)	700–800	0.3–0.8
AB-8	–	P(St-DVB)	480–520	0.3–0.8
ADS-21	Amide group	P(St-DVB)	80–100	0.3–0.8
D380	Amino group	P(St-DVB)	80–100	0.3–0.8
ADS-17	Ester group	P(St-DVB-MA)	90–120	0.3–0.8

Table 3
Purification results of different resins towards flavone compounds in medical plants.

Adsorbent	Purity of flavone compounds							
	Initial (%, w/w)				In product (%, w/w)			
	SB	HP	GB	RP	SB	HP	GB	RP
XAD-4	2.8	3.7	8.1	5.0	9.5	18.0	11.4	20.1
AB-8					14.2	18.5	12.8	28.6
ADS-21					16.5	24.1	30.8	30.1
D380					18.7	28.0	40.2	34.2
ADS-17					12.3	20.0	24.1	28.4

amino or amide groups on the adsorbents and phenolic hydroxyls in flavone molecule. It improved the selectivity of the adsorbents towards flavone compound. However, purification effects had not greatly improved and the degree of improvement in the purification ability for the different natural plants was very different. For example, purification effects of flavonoids in SB had not greatly improved compared to that of AB-8 or XAD-4, while purification effects of flavonoids in GB had been greatly increased, in that the matrix of D380 and ADS-21 which was also based on polystyrene having strong hydrophobic interaction, and hydrophobic impurities could still be adsorbed from natural plants. So it can hardly enhance the selectivity of flavonoids only through introducing functional group to hydrophobic matrixes of adsorbents.

Hydrophobicity of ADS-17 was weaker than above-mentioned resins owing to the participation of MA in its matrix. Ester group brought about the hydrogen bond. But because hydrogen bond between ester carbonyl and phenolic hydroxyl was so weak that adsorption selectivity to flavonoids could not be obviously improved.

Thus it is clear that the adsorbents without hydrogen group expressed poor selectivities to flavone compounds for their low pertinences to the structural characteristics of flavonoids. Introducing hydrogen group, which were able to form a hydrogen bond with phenolic hydroxyl in flavonoids, could increase their selectivity. However, the adsorption selectivity could not be fully expressed when hydrophobic force was too strong. Therefore, the hydrophobicity of adsorbents must be weakened when hydrogen bond groups were introduced. In this sense, EGDMA was selected as copolymerization agent in this study owing to its weaker hydrophobicity than polystyrene. In order to adjust the hydrophobicity of the adsorbents, a series of adsorbents with different EGDMA content and functional groups have been synthesized as described in Section 2.3.

3.2. Characterization of physical parameters of the synthesized adsorbents

Pore structure, moisture proportion and functional group content of the synthesized adsorbents were characterized as mentioned in Section 2.4, and the results were showed in Table 4.

There were no remarkable differences among the matrixes of the adsorbents by comparison on the specific surface area and average pore diameter. It illustrated that the hydropobicity of the adsorbents enhanced with the increase of the EGDMA content by the decrease of the moisture content of the synthesized adsorbents.

According to the amount of amino group listed in Table 4, amino groups were introduced to the adsorbent No. 1A–No. 5A successfully. And after acylation reaction, more than 95% of amino groups were changed to amide groups proved by the low residue amount of amino group of the adsorbent No. 1B–No. 5B.

3.3. Influence of functional groups on the adsorption properties of the adsorbents

Adsorption capacities of the adsorbents with different functional groups towards model compounds rutin and imperatorin were investigated, as shown in Fig. 2.

Adsorption capacity of the adsorbents No. 1–No. 5 with ester groups was low owing to their low hydrophobicities and weak hydrogen-bonding interaction with both rutin and imperatorin. By comparison, the adsorption capacity of No. 1A–No. 5A with amino groups and No. 1B–No. 5B with amide groups to rutin was obviously increased while nearly no changes in imperatorin. It was due to the fact that there was stronger hydrogen-bonding interaction between rutin and surface functional groups of the adsorbents, which compensated the weakened hydrophobic interaction and maintained the total adsorption driving force. The hydrogen-bonding interaction did not exist between the adsor-

Table 4
Pore structure, moisture proportion and functional group content.

Adsorbent	EGDMA content (%, w/w)	Particle size (mm)	Specific area (m^2/g)	Main functional group	Amount of amino group (mmol/g)	Moisture proportion (%, w/w)
No. 1	5	0.2–0.3	45	Ester	–	64.8
No. 2	10		61	Ester	–	62.5
No. 3	15		73	Ester	–	61.4
No. 4	20		82	Ester	–	60.8
No. 5	25		85	Ester	–	59.4
No. 1A	5		51	Amino	2.91	82.8
No. 2A	10		70	Amino	2.89	81.4
No. 3A	15		81	Amino	2.81	79.6
No. 4A	20		91	Amino	2.78	78.2
No. 5A	25		94	Amino	2.75	75.9
No. 1B	5		49	Amide	0.11	70.4
No. 2B	10		64	Amide	0.10	70.8
No. 3B	15		75	Amide	0.12	71.1
No. 4B	20		86	Amide	0.19	72.4
No. 5B	25		90	Amide	0.18	73.1

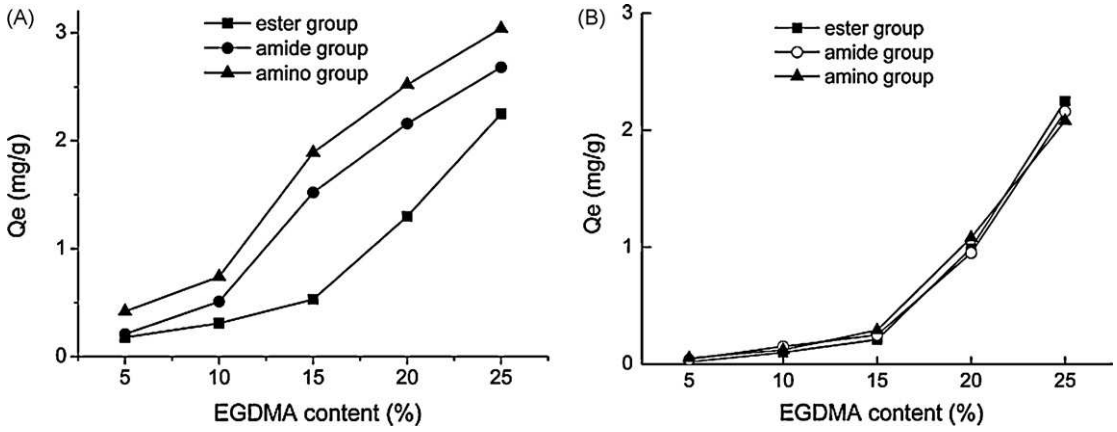

Fig. 2. Adsorption curves of adsorbents with different functional groups. (A) Adsorbate: rutin. (B) Adsorbate: imperatorin.

bents and imperatorin without phenolic hydroxyl groups like rutin, so there was no increase in the adsorption capacity. The result showed that the selectivity of the adsorbent towards flavonoids was enhanced remarkably after hydrogen groups were introduced onto the matrix of the adsorbent.

Desorption ratio of the adsorbent with different functional groups towards flavonoids was investigated and desorption curve was shown in Fig. 3. Considering the stability of flavonoids, aqueous ethanol solutions were used as desorption solutions. The desorption ratios of the flavonoids laden on the adsorbents No. 1A–No. 5A were rather lower than those laden on the adsorbents No. 1B–No. 5B, owing to the stronger hydrogen-bonding interaction between amino groups and phenolic hydroxyl groups than that between amide groups and phenolic hydroxyl. Certainly, using the ethanol aqueous solution with ammonia as eluent could improve the elution efficiency for the adsorbent No. 1B–No. 5B apparently, but under the alkaline conditions the flavone compound was unstable. Therefore, adsorbents with amide groups were most suitable for the purification of flavonoids among the synthesized adsorbents.

3.4. Influence of EGDMA content in adsorbent matrix on purification effect

Influence of the content of EGDMA in the adsorbent matrix on adsorption ability towards the model compounds rutin and imperatorin was investigated and the adsorption curve was illustrated in Fig. 4. When EGDMA content was less than 20%, the resin could hardly adsorb imperatorin owing to its weak hydrophobic affinity. Along with the increase in the EGDMA content, the adsorption capacity of the resin towards imperatorin increased obviously. But this influence was different towards the flavone compound rutin. Although the adsorbent No. 3B with 15% EGDMA content could not adsorb imperatorin only based on the hydrophobic affinity, it could adsorb rutin successfully owing to the assistance of hydrogen-bonding interaction. And then the adsorption ability of the adsorbent No. 4B, No. 5B with higher EDGMA content was improved accordingly. At the same time we also found that the adsorbents No. 1B, No. 2B with excessively low EGDMA content could not adsorb rutin, although contained the same amide group. It was because water molecule could hinder the hydrogen-bonding interaction between rutin and resins in aqueous solution. While the adsorbent had enough intensive hydrophobic force, it could help rutin overcome the interference from water molecules to approach the suitable location of adsorbent surface, which coincided with Schrader's research [22]. In light of the considerations presented above, No. 3B was the most appropriate one among No. 1B–No. 5B for its optimal selectivity to flavones compounds with acceptable adsorption capacity.

A natural plant, e.g. *S. barbata* D.Don (SB) flavonoids system, was selected to verify the above conjecture and the sample solution was prepared as described in Section 2.6. The dynamic adsorption and desorption tests were conducted as mentioned in Section 2.8. 80% ethanol aqueous solution (vol.%) was used as eluent. The purification results were listed in Table 5.

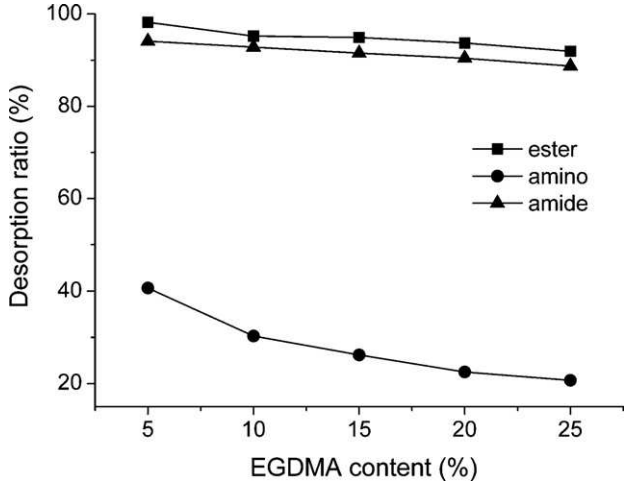

Fig. 3. Desorption curves of adsorbents with different functional groups towards rutin.

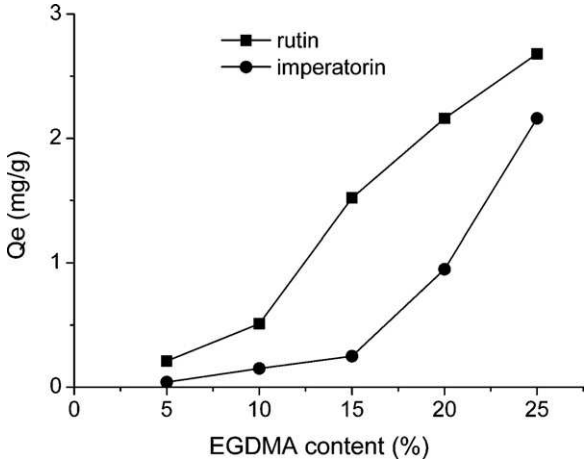

Fig. 4. Adsorption curves of adsorbents with different EGDMA content.

Table 5
Purification results of the adsorbents with different EGDMA content towards flavonoids in SB.

Adsorbent	EGDMA content (%, w/w)	Adsorption capacity (mg/mL)	Purity of flavonoids		Recovery (%)
			Initial content (%)	Content in product (%)	
No. 1B	5	0.45	2.8	–	–
No. 2B	10	0.91		54.6	60.1
No. 3B	15	1.45		52.7	96.8
No. 4B	20	1.53		39.6	95.5
No. 5B	25	1.68		35.4	94.8

Table 6
Adsorption isotherm parameter of No. 3B.

Temperature (K)	K_f	n	R^2
295	0.1344	0.7742	0.9968
303	0.1267	0.715	0.9907
311	0.0805	0.7577	0.9978

The relationship between the adsorption capacities towards SB flavonoids and EGDMA content was the same as the relationship shown in static tests discussed above. The purity of flavonoids in product was decreased gradually with the increase of EGDMA content, due to the enhancement of hydrophobicity of the adsorbents causing poor selectivity. The adsorbent No. 3B expressed preferable purification power among the synthesized adsorbents No. 1B–No. 5B.

3.5. Thermodynamics analysis

Thermodynamics for adsorption of flavonoids onto No. 3B was studied. Adsorption isotherms of No. 3B were carried out at 295 K, 303 K and 311 K, respectively. At the range of the concentration in this study, the adsorption isotherms were further correlated to the well known Freundlich equation. Freundlich equation parameters (K_f, n) were shown in Table 6. The n values were all less than 1. It indicated that the adsorption of rutin onto No. 3B resin took place easily [37].

The isosteric enthalpies of adsorption (ΔH_m) were calculated with a derivative Van't Hoff equation [38,39]. ΔH_m was calculated from the slope of line plotted by $\ln C_e$ against $1/T$. In Table 7, ΔH_m of rutin on No. 3B were shown corresponding to the different adsorption capacity. The negative values of all the enthalpies indicated an exothermic process. Isosteric adsorption enthalpies of No. 3B were in the range of adsorption enthalpies related on hydrogen-bonding interaction [40]. Accordingly, the adsorption process was considered to be related with hydrogen-bonding interaction.

3.6. Purification effects of No.3B towards flavonoids in other plants

Other three plants, GB, RP and HP, were selected to study the universality of the purification method established based on No. 3B. The results of purification and repeatability were listed in Table 8. No. 3B exhibited good purification effects on the flavonoids in the three plants selected. It illuminated that the method based on the synthesized macroporous adsorbent with amide group, No. 3B, was entirely practicable in purification of flavonoids in several plants. It was its predominance to commonly used methods based on commercial adsorbents.

Table 7
Isosteric adsorption enthalpies of rutin on No. 3B.

Q_e (mg/g)	K_0	ΔH_m (kJ/mol)	R^2
0.8	12.8	−25.0	0.9982
1.0	13.8	−27.1	0.9982
1.2	13.8	−26.5	0.9899

Table 8
Results of purification and repeatability in different plants.

Plant	Total flavonoids content			Recovery	
	Initial (%, w/w)	In product		% (w/w)	SD
		% (w/w)	SD		
HP	3.7	53.1	0.12	97.1	0.05
GB	8.1	59.6	0.05	93.2	0.08
RP	5.0	56.2	0.09	95.8	0.07
SB	2.8	52.5	0.03	93.6	0.09

4. Conclusion

In this study, a method based on macroporous polymeric adsorbents was established to purify the flavone compounds from several natural plants. According to the results based on the commercial adsorbents, a series of macroporous polymeric adsorbents with different hydrogen groups and hydrophobicity were synthesized, and the influences of functional groups and adsorbent matrix on the purification effect were studied. It is obvious that the hydrogen-bonding interaction between the flavonoids and the functional groups on the surface of adsorbents could be fully expressed with appropriate hydrophobic forces intensity. The flavone compounds in natural plants could be adsorbed selectively due to the synergistic interaction of proper hydrogen-bonding and hydrophobic interaction. In this paper, the adsorbent No. 3B with 15% EGDMA content and amide group performed the most appropriate power to purify flavonoids from the four chosen plants owing to its proper hydrophobicity and hydrogen-bonding groups. Adsorption thermodynamics analysis showed that isosteric adsorption enthalpies of No. 3B were in the range of 25–30 kJ/mol, which was in the range of adsorption enthalpies related to hydrogen-bonding interaction. The universality of the method could find its full expression via its good effects in the purification of total flavonoids from SB, GB, RP and HP, and the purity of flavonoids in final products was above 50%, which testified that the synthesized adsorbents were superior to the commercial adsorbents. The two step adsorption–desorption process was suitable for mass production for its simple procedure, low cost, and high universality.

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REBRAPA
REVISTA BRASILEIRA DE PESQUISA EM ALIMENTOS

Atualidades sobre a química e a utilização do urucum (Bixa orellana L.)

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Resumo: A cor dos alimentos é o primeiro atributo avaliado pelo consumidor. Por isso, há a preocupação da indústria de alimentos em adicionar corantes aos produtos para torná-los mais atraentes. Entre os corantes utilizados na indústria de alimentos, o urucum é uma das matérias-primas da qual pode-se obter diversos tipos de pigmentos naturais. O corante de urucum é extraído da camada externa das sementes de Bixa orellana L. por imersão em solução alcalina, óleo vegetal ou solventes orgânicos. Além das preparações comerciais, mais da metade das sementes de urucum produzidas no Brasil são utilizadas no preparo do colorífico, a partir da mistura do pigmento ou da semente triturada com farinha de milho, óleo vegetal e sal. Além do principal pigmento do urucum, o carotenoide bixina, suas sementes possuem outros componentes, como o geranilgeraniol, que apresenta importantes propriedades farmacológicas. A variabilidade do corante de urucum pode ser influenciada por condições de pós-colheita, processamento e emprego de diferentes cultivares na sua fabricação. Após a industrialização do urucum, são gerados em média 96% de resíduos, que depois de secos e triturados podem ser reutilizados. Já durante a análise do pigmento presente em alimentos, as técnicas de preparo, separação e quantificação requerem, na maioria das vezes, o uso de padrões com alta pureza e estabilidade.

Palavras-chave: Bixa orellana; bixina; CLAE; estabilidade; subproduto.

Updates on chemistry and use of annatto (Bixa orellana L.): Food color is the first attribute evaluated by the consumer. Therefore, the food industry is concerned about the use of colorants in food materials to make them more attractive. Between the colorants used by the food industry, annatto is a raw material that allows the obtaining a large amount of natural pigments. The annatto dye is extracted from the outer layer of Bixa orellana L. seeds by immersion in alkaline solution, vegetable oil or organic solvents. In addition to the commercial preparations, a large amount of brazilian annatto seeds is used to prepare "colorífico", a spice obtained from the mixture of pigment or grinded seeds with corn flour, vegetable oil and salt. Besides the bixin carotenoid, the seeds presents other components such as geranylgeraniol, with important pharmacological properties. The annatto seeds variability are influenced by conditions of post-harvest processing and the different cultivars used in industry. After the annatto processing, about 96% of waste are generated, which after dried and milled remain appropriate for reuse. During de pigment analysis in foodstuffs, the preparation techniques, the compounds separation and quantification requires, the use of standards with high purity and stability.

Keywords: Bixa Orellana; bixin; HPLC; stability; by-product.

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INTRODUÇÃO

Quase 90% das percepções dos sentidos do ser humano acontecem através da visão. A luz, sob diferentes comprimentos de onda, quando penetra nos olhos provoca estímulos no cérebro e produz as distinções de cor (ANGELUCCI,

1988). A cor desempenha um papel na escolha dos alimentos através da influência nas percepções de sabor, preferência, satisfação e aceitabilidade, interferindo em julgamentos de intensidade e identificação de sabor (CLYDESDALE, 1993).

Apesar de subjetiva, a aceitação de um produto alimentício pelo consumidor está diretamente ligada com a cor, sendo este o primeiro atributo a ser avaliado. Por isso, existe a preocupação das indústrias de alimentos em adicionar corantes aos produtos como forma de restituir a cor original perdida durante o processamento, tornar um alimento mais atraente, relacionando a cor a outras características como sabor e aroma, conferir cor a alimentos incolores ou somente reforçar cores existentes (CONSTANT; STRINGHETA; SANDI, 2002).

Os corantes disponíveis para utilização em alimentos podem ser divididos em dois grupos, os artificiais e os naturais. Apesar dos corantes artificiais serem mais baratos e possuírem maior estabilidade, existe uma tendência de substituição de aditivos sintéticos em geral, pelos ingredientes naturais. Além da tendência de consumo, também existe a atribuição de propriedades funcionais a alguns desses corantes naturais (CONSTANT; STRINGHETA; SANDI, 2002; ZOU; AKOH, 2015).

A denominação "natural" não significa necessariamente que o produto é bom ou saudável. Os corantes naturais, da mesma forma que os artificiais, necessitam de especificação de pureza, de restrição de uso do estabelecimento de quantidades máximas permitidas pelos órgãos competentes (Angência Nacional de Vigilância Sanitária, Food and Drug Administration e Comunidade Europeia). A substituição do uso dos corantes sintéticos pelos naturais, apesar de tratar-se de uma tendência, nem sempre ocorre e requer desenvolvimento de tecnologias e métodos de extração que garantam suas propriedades, além de métodos analíticos confiáveis para determinação (SCOTTER, 2011).

No Brasil, uma das principais matérias-primas utilizadas na produção de corantes naturais é o urucum. O pigmento do urucum é extraído da camada externa das sementes da planta de Bixa orellana L. sendo fonte do carotenoide bixina (STRINGHETA; SILVA, 2008).

A bixina é indexada no Colour Index, um órgão internacional de nomenclatura de corantes, como CI n°75120, mas a denominação mais conhecida é da Comunidade Europeia, como ECC n°E160b (MARMION, 1991; OLIVEIRA, 2005).

O objetivo deste trabalho de revisão de literatura foi abordar os principais aspectos relacionados ao urucum utilizado como corante natural em alimentos, desde as características da espécie, cultivo, colheita, pós-colheita e as principais técnicas de processamento, até as características químicas e métodos de análise dos pigmentos. Também foi comentado sobre alguns estudos recentes envolvendo componentes minoritários, que apresentam potencial de utilização, apesar de pouco conhecidos, e as alternativas de reaproveitamento do principal resíduo da produção do corante.

MATERIAIS E MÉTODOS

Neste trabalho, foi realizado um levantamento bibliográfico sobre o corante natural de urucum utilizado na indústria de alimentos. Para a pesquisa, foram utilizadas as bases de dados Scielo e Science Direct. Também foi utilizada a Biblioteca Digital da UNICAMP, Biblioteca do Instituto de Tecnologia de Alimentos (ITAL), além de anais de congressos e simpósios da área de alimentos e específicos sobre a cadeia produtiva do urucum. Foram utilizadas palavras-chave em inglês e português, entre elas: Bixa orellana, annatto, urucum, bixina, bixin, carotenoids, geranylgeraniol, HPLC, chromatographic standard e byproducts. Algumas informações foram foram atualizadas em novembro de 2014.

O artigo foi estruturado em uma sequência lógica que abordou inicialmente o urucum, seu principal corante, a bixina e os outros componentes encontrados na semente. Em seguida, foram tratados dos principais métodos analíticos para determinação da bixina, as técnicas de processamento das sementes e do resíduo da industrialização.

O urucum

O urucuzeiro é originário da América Tropical, pertence à família Bixaceae com o nome botânico de Bixa orellana L. (GOUVEIA; MOURA; MEDEIROS, 2000). Dependendo da região de cultivo e da idade da planta, apresenta-se como um arbusto perene grande ou como uma árvore pequena, variando de 2 a 5 metros de altura. A planta exibe grande variabilidade de coloração, com caule, frutos verdes e flores brancas ou caule vermelho, flores rosas e frutos vermelho-escuro (Figura 1) (INGRAM; FRANCIS, 1969).

Os frutos são do tipo cápsula ou cachopa, ovoides ou globosos, com 2 a 3 carpelos que variam de 3 a 4 cm de comprimento e 3 a 4,5 cm de diâmetro. Externamente, são revestidos por espinhos moles e possuem coloração variável entre o verde, vermelho-pálido e roxo. No interior, estão normalmente divididos em duas valvas com um conteúdo de grãos que varia de 10 a 50 (Figura 1) (INGRAM; FRANCIS, 1969; PRESTON; RICKARD, 1980).

Os grãos são arredondados, revestidos por uma camada pastosa de coloração avermelhada, os quais tornam-se secos, duros e de coloração escura com o amadurecimento (Figura 2). Apresentam diâmetro médio de 0,4 cm. A bixina é o pigmento presente em maior concentração nos grãos, representando mais de 80% dos carotenoides totais do urucum, lipossolúvel e sujeita à extração com alguns solventes orgânicos. (FRANCO et al., 2002).

De acordo com Franco et al. (2008), o urucuzeiro floresce, frutifica e matura durante, praticamente, todo o ano. No Paraná, em condições normais de clima, a primeira floração é mais intensa entre os meses de fevereiro e março, cuja colheita principal ocorre de junho a julho. A segunda floração ocorre nos meses de julho e agosto com colheita em novembro e dezembro, sendo ambas as colheitas executadas geralmente de forma manual, já que a maior parte da produção do urucum é proveniente da agricultura familiar.

As cápsulas devem ser colhidas apenas quando estiverem maduras e secas, pois o elevado percentual de umidade dos grãos contribui para o crescimento de micro-organismos. Os frutos colhidos permanecem no campo por um curto período de tempo, no espaço conhecido como entrelinhas das plantas e no caso de chuva, são recolhidos em local coberto (FRANCO et al., 2002).

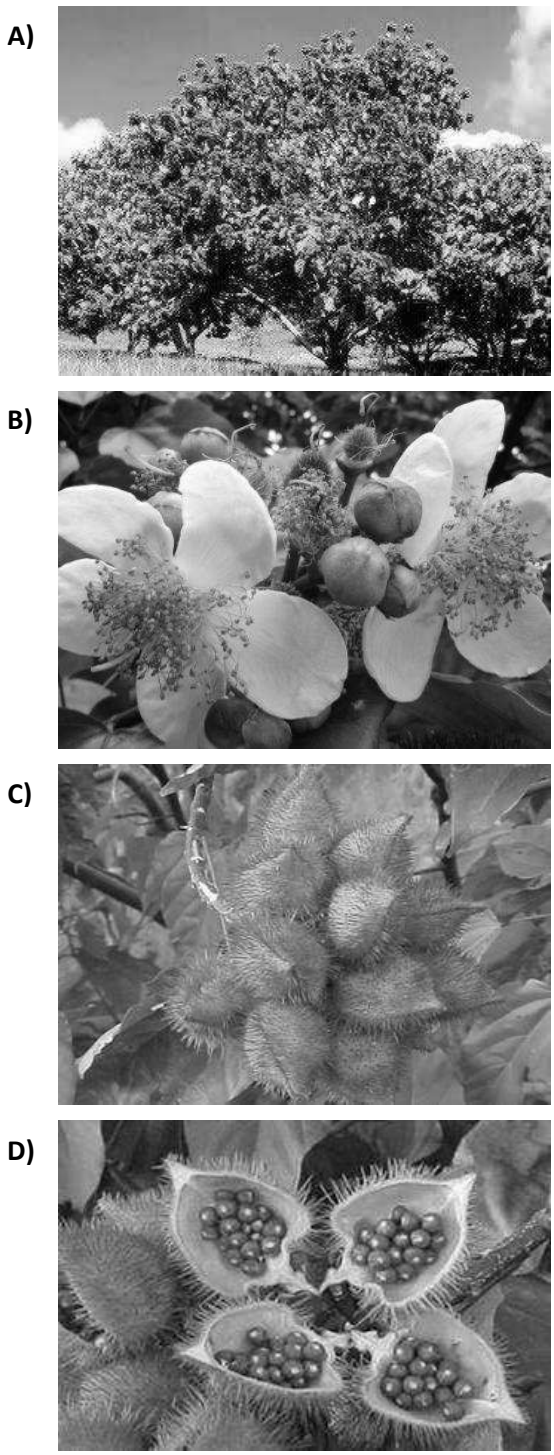

Figura 1- A) Planta de urucuzeiro; **B)** Floração de urucuzeiro; **C)** Frutos de urucuzeiro; **D)** Sementes de urucuzeiro. Fonte: FRANCO et al., (2002).

Figura 2- Frutos de urucuzeiro em maturação plena.

O descachopamento, operação conhecida como a separação das sementes das cachopas, pode ser efetuado manual ou mecanicamente, sendo que este último é o mais indicado, por apresentar menores perdas de corante. A secagem consiste no recolhimento dos grãos peneirados sobre lonas, em terreiros ou secadores de alvenaria durante aproximadamente um dia (FRANCO et al., 2002).

Após o beneficiamento e principalmente durante o armazenamento, observa-se que a diminuição do teor de corante está associada a alguns fatores, tais como teor de umidade, atividade de água dos grãos, temperaturas elevadas, exposição ao ar e umidade relativa do ambiente, conforme citado por Corrêa et al. (1991).

No Brasil, a cultura do urucum ainda não dispõe de variedades, mas da denominação de cultivares. Os principais tipos cultivados de interesse para as principais regiões produtoras são: Peruana Paulista (São Paulo), Bico de Pato (Bahia), Piave Vermelha (Pará), Piave Vermelha Grande, Bico de Calango, Verde Amarela, entre outros cultivados nas demais regiões do país (REBOUÇAS; SÃO JOSÉ, 1996).

A cultivar Piave, se plantada no Nordeste brasileiro, produz grãos com teor de bixina em torno de 1,75% (FRANCO et al., 2002). Em contrapartida, em solo paranaense e lavouras adequadamente conduzidas, a cultivar Piave produz grãos com até 6% de teor de bixina (FRANCO et al., 2008).

No Sul do Brasil, o estado do Paraná é o maior produtor de urucum da região. A cultura paranaense, que teve início na década de 1980, está concentrada nos municípios de Paranacity e Cruzeiro do Sul, na região Noroeste (RÜCKER; MORSBACH, 1996; FRANCO et al., 2008). De acordo com um levantamento realizado pela Secretaria da Agricultura e do Abastecimento do Estado do Paraná, são verificadas oscilações tanto na produção estadual quanto no preço pago ao agricultor (Tabela 1).

Tabela 1 – Produção, área e valordo Urucum no Paraná, de 2005 a 2010.

Ano	Produção (Kg)	Área (Ha)	Valor (R$)	
			Total	Kg
2005	2.763.844	1.099,0	3.786.466,28	1,37
2006	1.261.680	1.092,5	2.371.958,40	1,88
2007	1.171.844	1.052,3	2.542.901,48	2,17
2008	1.142.138	1.042,9	2.969.558,80	2,60
2009	1.340.350	1.155,2	3.699.366,00	2,76
2010	1.467.019	1.279,8	3.828.919,59	2,61

Fonte: PARANÁ, 2012.

Para efeitos comerciais, consideram-se como parâmetros de qualidade a umidade das sementes, o teor de bixina, o odor típico, a presença de impurezas, material estranho e mofo (FRANCO et al., 2008). Na Tabela 2 é apresentada uma classificação das sementes, de acordo com os atributos de qualidade, proposta por Franco et al. (2002).

O teor de pigmentos presentes nas sementes de urucum oscila de acordo com a variedade da cultura, do solo, do clima e dos tratos culturais, podendo ser encontradas sementes com menos de 1% e outras até com 6% de bixina (CARVALHO; HEIN, 1989).

Mazzani, Marin e Segovia (2000) analisaram dez cultivares diferentes de urucum em uma coleção na Venezuela e verificaram que a procedência das plantas analisadas foi responsável pela diferença entre elas. Carvalho et al. (2010) explica que os resultados obtidos em estudos comparativos fornecem informações

que podem ser utilizadas em trabalhos que buscam o melhoramento genético a partir da combinação de plantas com as características adequadas a cada finalidade (CARVALHO et al., 2010).

Tabela 2 – Classificação comercial das sementes de urucum.

Fatores de qualidade	Classe		
	Tipo 1	Tipo 2	Tipo 3
Umidade	≤10%	10% a 14%	>14%
Bixina	>2,5%	2% a 2,5%	<1,8%
Impurezas	<5%	<5%	>5%
Materiais estranhos	Ausência	Ausência	Presença

Fonte: FRANCO et al. (2002).

Entretanto, as dificuldades na comparação entre os resultados do teor de bixina de diferentes procedências e variedades podem estar relacionadas à falta de uniformidade dos métodos de análise. As metodologias usadas variam desde a utilização de diferentes soluções para extração dos pigmentos até o uso inadequado de coeficientes de absorção para quantificação (CARVALHO et al., 2010).

Bixina

A bixina é o corante do urucum responsável pelas tonalidades que variam do amarelo ao vermelho ($C_{25}H_{30}O_4$), um diapo-carotenoide, representado pela parte central da molécula de um carotenoide, sem os anéis terminais característicos da maioria dos compostos desta classe (Figura 3) (STRINGHETA; SILVA, 2008).

A bixina apresenta a particularidade dentre os carotenoides por ser encontrada naturalmente na configuração cis e por possuir em sua molécula dois grupos carboxílicos, sendo um deles um éster metílico. Esta característica confere lipossolubilidade à molécula. Se ocorrer a hidrólise alcalina do agrupamento metílico, obtém-se o sal hidrossolúvel da norbixina (SILVA, 2007).

Figura 3- Estrutura química da bixina.

Fonte: STRINGHETA; SILVA (2008).

O extrato de urucum tem uma estabilidade considerável à oxidação pelo oxigênio em meio anidro, mas uma resistência mais baixa aos efeitos da luminosidade. Uma forma de garantir a estabilidade do corante sob a incidência luminosa é a adição de antioxidantes, conforme sugeriram Najar, Bobbio e Bobbio (1988). Kiokias e Gordon (2003), investigaram as propriedades antioxidantes dos carotenoides de urucum em emulsões oleosas comestíveis. Os autores também pesquisaram a sinergia entre os pigmentos e outros antioxidantes naturais adicionados às emulsões e verificaram que a norbixina apresentou efeito sinergístico com tocoferóis e ácido ascórbico, retardando a deterioração oxidativa dos lipídios.

Ao analisarem extratos de urucum obtidos com solventes de diferentes polaridades (água, etanol/água, etanol, etanol/acetato de etila e acetato de etila), Chisté, Benassi e Mercadante (2011) encontraram uma correlação positiva entre os teores de bixina e parâmetros de cor (L* e C*) dos extratos. Os autores ainda verificaram correlação entre teores de bixina e atividade antioxidante in vitro, determinada pelos métodos de ABTS e porcentagem de proteção contra o oxigênio singlete. O extrato obtido com solução etanol/acetato de etila apresentou os maiores níveis de atividade antioxidante e a maior porcentagem de proteção contra a ação do oxigênio singlete. E por outro lado, os solventes acetato de etila e solução etanol/água foram os menos efetivos para extração de compostos fenólicos e bixina, respectivamente.

Cardarelli, Benassi e Mercadante (2008), estudaram extratos de urucum obtidos com diferentes solventes (metanol, etanol,

metanol/água, etanol/água, acetato de etila e hexano). As autoras verificaram correlação positiva entre o teor de bixina e a coloração vermelha. Também foram avaliadas as propriedades antioxidantes dos extratos pelo método de ABTS e foi observada correlação com os teores de compostos fenólicos totais, medidos pelo método de Folin-Ciocalteu. Apesar do estudo apontar os melhores resultados com o uso de solventes de média polaridade, como o metanol, deve ser considerado o potencial tóxico do solvente ao se tratar da aplicação em alimentos ou cosméticos.

Outros componentes

Juntamente com os carotenoides, os compostos fenólicos são considerados importantes para a saúde humana, por serem responsáveis por algumas funções biológicas como a diminuição do risco de doenças inflamatórias, degenerativas e cardiovasculares (KRINSKY, 1994). Chisté et al. (2011) identificaram compostos fenólicos como hipoaletina e derivados de ácido cafeico em sementes de urucum.

Mas apesar de bastante difundidas na cultura popular, as propriedades medicinais da planta do urucuzeiro ainda são pouco estudadas. Desde a utilização pelos índios, como proteção contra queimaduras solares e repelente de insetos, existem relatos da utilização das partes da planta na forma de chá, maceradas ou como xarope, no tratamento de febre, queimaduras, como cicatrizante, diurético, antialérgico e até como antídoto antiofídico (MORAIS et al., 2005; STRINGHETA; SILVA, 2008).

Algumas pesquisas dão suporte científico para o uso popular do urucum, como o estudo conduzido por Coelho et al. (2003). Os autores verificaram que tinturas extraídas do caule, flor, folha, fruto e raiz de exemplares de urucum inibiram o desenvolvimento de algumas espécies de bactérias estudadas, entre elas: Candida albicans, Enterococcus faecalis, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus sp e Salmonella sp. Irobi, Moo-Young e Anderson (1996), verificaram atividade antimicrobiana de um extrato etanólico frente algumas bactérias gram positivas, como Bacillus subutillis, Staphylococcus aureus e Streptococcus faecalis. Gonçalves, Alves Filho e Menezes (2005) constataram que o extrato hidroetanólico apresentou atividade antimicrobiana contra Streptococcus pyrogens, Proteus mirabilis e Staphylococcus aureus. E Majolo, Carvalho e Wiest (2013) concluíram que, entre as bactérias estudadas, a maior sensibilidade ao extrato de urucum foi verificada em Enterococcus faecalis e Listeria monocytogenes, enquanto que a enterobactéria Escherichia coli mostrou-se menos sensível.

Apesar da bixina ser o único componente do urucum que apresenta importância comercial, a planta possui vários compostos exóticos, a maioria recentemente identificada e alguns deles não são encontrados em nenhuma outra planta (VILAR et al., 2014). Entre eles, o geranilgeraniol (Figura 4), é encontrado na parte externa da semente, sendo o óleo essencial da semente do urucum a fonte mais abundante deste componente, com teores próximos a 1% (COSTA; CHAVES, 2005; STRINGHETA; SILVA, 2008).

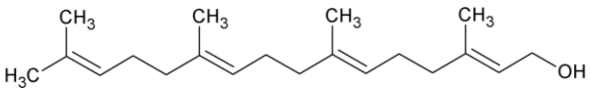

Figura 4- Estrutura química do geranilgeraniol.

Fonte: STRINGHETA; SILVA (2008).

De acordo com Jondiko e Pattenden (1989) e Silva et al. (2010), o geranilgeraniol é utilizado como um importante intermediário de biossínteses de substâncias como a vitamina K, de tocoferóis e tocotrienóis, de diversos hormônios e carotenoides.

Baseados na escassez de trabalhos tratando do geranilgeraniol em urucum, Silva et al. (2011) propuseram uma metodologia para extrair e avaliar a concentração deste composto em sementes produzidas no estado de São Paulo. No estudo, foram reportados valores que variaram de 0,32% a 1,38% de geranilgeraniol, apresentando uma correlação positiva com os teores de lipídios totais nas amostras.

Métodos de determinação da bixina

Método espectrofotométrico

Os primeiros métodos de análise de carotenoides em sementes de urucum baseavam-se em determinações espectrofotométricas (YABIKU; TAKAHASHI, 1991; CARVALHO; SILVA, MOREIRA, 1993).

Para o cálculo dos resultados, é necessária a utilização dos coeficientes de extinção ou absorbância. Dependendo do coeficiente utilizado, podem aparecer discrepâncias entre os resultados. Vários autores já publicaram coeficientes de extinção para bixina e norbixina e são verificadas diferenças significativas entre eles e os resultados encontrados (STRINGHETA; SILVA, 2008).

Apesar de ser um método rápido e barato, no caso da utilização da espectrofotometria para determinação da bixina, existe o inconveniente de que os isômeros apresentam comprimentos de onda próximos aos do carotenoide original. Isto dificulta a verificação de sua presença apenas através da leitura dos carotenoides totais (RIOS, 2004). Por este motivo, é recomendada a utilização da Cromatografia Líquida de Alta Eficiência (CLAE) para separação dos compostos.

Cromatografia líquida de alta eficiência

Além da vantagem da separação dos isômeros, os métodos cromatográficos por CLAE permitem o monitoramento da análise simultaneamente, em diferentes comprimentos de onda, com o uso de pequenas alíquotas de amostras (STRINGHETA; SILVA, 2008).

Diversos autores propõem metodologias para detectar, separar e quantificar pigmentos de urucum em alimentos (GLÓRIA; VALE; BOBBIO, 1995; MERCADANTE; PFANDER, 2001; TOCCHINI; MERCADANTE, 2001; RIOS, 2004; RIOS; MERCADANTE, 2004; MONTENEGRO et al., 2004; CARDARELLI; BENASSI; MERCADANTE, 2008; STRINGHETA; SILVA, 2008). A escolha do método de extração do corante da matriz alimentícia, ou da detecção, separação e quantificação do pigmento dependem da composição e da complexidade da matriz alimentícia, além do tempo e do custo para cada método (RIOS, 2004; STRINGHETA; SILVA, 2008).

Ao pesquisarem o teor de bixina por CLAE em colorífícos, Tocchini e Mercadante (2001) utilizaram um método de extração do pigmento que consiste em extrações sucessivas de cerca de 0,3 g de amostra em metanol e acetona (50 mL), em ultra-som, antes da injeção no cromatógrafo. As autoras separaram os carotenoides em um cromatógrafo a líquido de alta eficiência com fase móvel composta de acetonitrila:ácido acético 2% (65:35), volume de injeção de 20 μL, fluxo de 1 $mL.min^{-1}$, coluna C18 150 mm x 4,6 mm e detector de arranjo de diodos operando a 470 nm. O método proposto foi considerado preciso, exato e prático.

A CLAE também pode ser utilizada em análises das sementes de urucum. Silva et al. (2010) validaram uma metodologia para determinação de bixina e norbixina em urucum, que consiste na pesagem de 10 grãos inteiros (cerca de 0,28 g) seguida de extrações sequenciais do pigmento utilizando 10 mL de clorofórmio em banho ultra-som até completar um balão volumétrico de 250 mL seguido de secagem de uma alíquota de volume conhecido do extrato sob N_2 para diluição em fase móvel composta por acetonitrila:metanol:clorofórmio:ácido acético 6% (60:20:10:10) antes da injeção no cromatógrafo a líquido. Os autores sugerem ainda como condições cromatográficas uma vazão de 1 mL min^{-1}, coluna de 250 mm x 4 mm e 5 μm e monitoramento a 460 nm. O tempo de análise foi estabelecido em 6 minutos e o método apresentou exatidão.

Padrão cromatográfico

A maior dificuldade nas análises de carotenoides por CLAE é obter e manter padrões puros. Os carotenoides altamente insaturados são suscetíveis à isomerização e oxidação. Mesmo com poucos padrões de carotenoides disponíveis comercialmente (alfa-caroteno, beta-caroteno e licopeno, por exemplo), eles são caros, principalmente se há necessidade de importação. Portanto, é útil para um laboratório de análise de carotenoides o

desenvolvimento e a prática de isolar e manter seus próprios padrões, inclusive aqueles que não podem ser obtidos comercialmente (RODRIGUEZ-AMAYA, 2001; KIMURA; RODRIGUEZ-AMAYA, 2002).

Quando um laboratório adquire um padrão comercial, isso pode acontecer vários meses após ele ter sido produzido e o tempo entre a produção do padrão e a sua compra. Tal situação pode trazer diferenças na pureza verificada. E ainda, as condições de armazenamento recomendadas demonstram a variabilidade de acordo com diferentes fornecedores, como é o caso da temperatura de estocagem indicada pelo fornecedor, que pode variar de temperaturas negativas até a temperatura ambiente (AVRAMIDES, 2005).

Processamento das sementes de urucum

De acordo com Preston e Rickard (1980) e Carvalho (1999), existem três processos comerciais para extração do pigmento dos grãos de urucum. A mais utilizada é a extração por imersão em solução alcalina, e em seguida a extração por imersão em óleo vegetal e em solventes orgânicos. Além das preparações comerciais utilizadas como corantes, mais da metade das sementes de urucum produzidas no Brasil são usadas para o preparo de uma especiaria conhecida como colorífico, totalmente consumida no mercado nacional. O colorífico é obtido a partir da mistura do pigmento ou da semente triturada do urucum com farinha de milho, óleo vegetal e sal (GHIRALDINI, 1989; CARVALHO, 2010b).

Na industrialização dos corantes de urucum, as soluções alcalinas, como hidróxido de sódio ou potássio, convertem a bixina da sua forma lipossolúvel a hidrossolúvel, através da hidrólise alcalina formando o ácido dicarboxílico livre, a norbixina (CARVALHO, 1992; FRANCO et al., 2002).

A extração direta do pigmento, pela imersão da semente em óleo vegetal comestível refinado produz uma solução oleosa de bixina, que é aquecida e depois filtrada. O extrato é utilizado em alimentos com alto teor de lipídios e apresenta colorações variadas de acordo com as temperaturas de extração utilizadas, que acabam dando origem a corantes alaranjados ou amarelos, resultado da formação de isômeros mais estáveis (PRESTON; RICKARD, 1980; STRINGHETA; SILVA, 2008).

Na extração com solventes orgânicos, existe a limitação de acordo com a necessidade de um solvente compatível com a utilização no alimento. Existem sérias restrições devido à toxidade de determinados solventes que restringem o seu emprego no processamento (STRINGHETA; SILVA, 2008).

A partir dos inconvenientes de alguns métodos tradicionais, surgem processos alternativos, como tecnologias limpas ou métodos inovadores. É o caso da extração supercrítica, que utiliza um fluido em condições críticas de temperatura e pressão, um processo atóxico e que não deixa resíduos nos extratos obtidos (SILVA; CABRAL, 2000; PESSOA et al., 2006).

Barreto, Jaeger e Massarani (1989) propuseram o uso de atrito mecânico das sementes em moinhos de bolas para extração da bixina e Massarani, Passos e Barreto (1992) e Shuhama et al. (2003) estudaram o emprego de leitos de jorro na obtenção do corante.

Outro método, relatado por Carvalho (2010a), consiste na extração do corante de urucum utilizando apenas água como solvente. O autor cita a vantagem da manutenção das características do pigmento em um processo simples, seguro, com resíduo de baixo impacto. É sugerido ainda, que a técnica possa ser desenvolvida em instalações próximas aos produtores das sementes. A facilidade de armazenamento e do transporte dos pigmentos até as indústrias de corantes eliminaria a geração do resíduo das sementes esgotadas, que representa um problema para as indústrias processadoras do corante e poderia ser reaproveitado pelos próprios produtores rurais (GUIMARÃES; BARBOSA; MASSARANI, 1989; CANTO et al., 1991; CARVALHO, 2010a).

Farelo da semente de urucum

Considerando que o grão de urucum possui, no máximo, cerca de 6% de bixina, pode-se afirmar

que a extração industrial do pigmento ocasiona cerca de 94% de sobras que, descartadas pela indústria, podem poluir o meio ambiente (SILVA et al., 2006).

Como alternativa na minimização de resíduos, o material pode passar por um processo de secagem e ser utilizado como adubo em plantações, suplemento de ração animal e ainda agente de pigmentação de gemas de ovos (UTIYAMA, 2001).

De acordo com Bressani et al. (1983), o resíduo das sementes caracteriza-se por possuir um alto teor de proteínas (13% a 17%), fibra bruta (aproximadamente 16%) e alto teor de fósforo. No total das proteínas presentes, os autores encontraram níveis nutricionalmente adequados dos aminoácidos triptofano e lisina. Demczuk Jr. et al. (2010) encontraram níveis significantes do carotenoide bixina no farelo de semente de urucum.

O potencial de utilização do farelo é comprovado por alguns autores que já estudaram o efeito do resíduo da semente processada e também de extratos de urucum na alimentação animal. Harder et al. (2010) relataram que o uso de urucum resultou em um aumento na pigmentação de cortes de peito de frango. Utiyama (2001) estudou a viabilidade do uso do farelo da semente de urucum adicionado na ração de suínos, como um ingrediente alternativo ao milho e ao farelo de soja. O autor verificou que o farelo pode ser substituído em níveis de até 10% na ração, sem prejudicar o desempenho de suínos em fase de crescimento. Queiroz (2006) avaliou o efeito do farelo da semente de urucum como agente de pigmentação de gema de ovo de galinhas poedeiras comerciais.

CONSIDERAÇÕES FINAIS

A tendência da utilização de produtos naturais, com características funcionais ou apelo saudável, faz do urucum uma matéria-prima importante para a produção de corantes alimentícios, já que o Brasil é um dos maiores produtores mundiais de sementes de urucum e o estado do Paraná tem relevante representatividade no mercado nacional.

O principal pigmento do urucum, o carotenoide bixina, é extraído da camada externa das sementes de Bixa orellana L. por imersão em solução alcalina, óleo vegetal ou solventes orgânicos. A diversidade de produtos que podem ser obtidos a partir das sementes de urucum é útil para satisfazer a necessidade de aplicação em vários tipos de alimentos industrializados. O conhecimento das características das sementes de diferentes cultivares serve para identificar e valorizar uma determinada região produtora, além de auxiliar no desenvolvimento de novas tecnologias de extração ou no aprimoramento daquelas já existentes.

Após a extração do pigmento, as sementes de urucum são descartadas, podendo representar problemas ambientais. No entanto, se reutilizadas como ingrediente de ração animal pela associação às suas propriedades químicas, físicas e funcionais, podem contribuir para diminuição do desperdício.

Os grãos de urucum ainda possuem outros componentes de importância, mas pouco explorados, como o geranilgeraniol, que apresenta importantes propriedades farmacológicas.

Considerando as transformações químicas às quais os carotenoides estão expostos durante o processamento de um alimento, vários estudos já investigaram os mecanismos de degradação dos pigmentos do urucum, principalmente pelo uso de metodologias analíticas que empregam a CLAE. Porém, no que diz respeito às determinações em laboratório, ainda existe um potencial de exploração de como os padrões cromatográficos podem ser mantidos com a estabilidade e pureza necessária.

De uma forma geral, ao serem verificadas publicações utilizadas nesta revisão, pode-se afirmar que existe uma extensa e consolidada base de dados sobre o urucum. Os trabalhos mais antigos abordando a semente, tratam principalmente dos aspectos tecnológicos de processamento do corante e as características químicas de seus principais constituintes. Nos trabalhos mais recentes, como os da última década, a atenção voltou-se às características funcionais de extratos de urucum. Já nos

trabalhos atuais, além das propriedades funcionais continuarem sendo alvo de estudos, é verificada uma tendência à exploração de novos compostos identificados no urucum, que possuem potencial farmacológico difundido apenas pela sabedoria popular. E aliadas aos conceitos de inovação e sustentabilidade, as chamadas "tecnologias limpas" de extração dos compostos de interesse, surgem com o objetivo de utilizar solventes baratos, de fácil recuperação e baixa ou nenhuma toxidade.

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Bioremediation potential of natural polyphenol rich green wastes: A review of current research and recommendations for future directions

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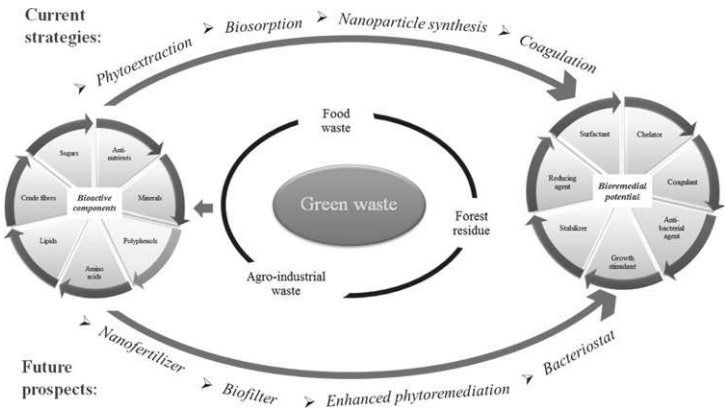
HIGHLIGHTS

- Food, agro-industrial and forest residues are the renewable sources of polyphenols.
- Total polyphenolic content of green waste range from 0.01 to 925 mg g^{-1} dry extract.
- Green waste offers eco-friendly and economical route to synthesize nanoparticles.
- Bioremedial traits of green wastes are biosorption, phytoextraction and coagulation.
- Natural polyphenol based nanoamendments or filters could be developed in the future.

GRAPHICAL ABSTRACT



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ABSTRACT

'Green waste' (food, agro-industrial and forest residues) is a renowned valuable resource of polyphenols. Natural polyphenols are relatively efficient in the clean-up of environmental pollutants based on their unique traits of chelation, adsorption, reduction, complexation, nutrient cycling, antibacterial effects and plant growth promotion. These significant traits have found emerging applications in the removal of heavy metals, pathogenic bacteria and dyes from contaminated soil and water through existing bioremedial techniques such as biosorption, phytoextraction and coagulation. Increasingly, polyphenol-rich natural extracts harnessed for green nanoparticle synthesis (production of particles between 1 and 100 nm in size using biological entities such as microorganisms or plant biomass) have found promising use as a remedial agent in the detoxification of toxic pollutants. However, current bioremediation approaches do not sufficiently exploit natural polyphenols, which are abundantly available and are non-toxic. This review examines the extent of natural polyphenol availability in green waste, and provides a critical view on the existing remedial options, knowledge gaps and hence scope for future research. It highlights the use of natural polyphenol-rich green wastes as nanofertilizers, bioamendments, biofilters and bacteriostats. Field application strategies such as microbe-assisted phytoremediation, bioaugmention and biostimulation are also emphasized, showing the multifunctional biotechnological potentials offered by natural polyphenols.

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Contents

1. Introduction

Intensive urbanization and industrialization, in addition to population pressure on limited natural resources, and to some extent the natural activities, have all posed immense stress to the global environment by generating toxic contaminants. The focal tool of the USEPA is to set out a suitable remediation strategy against environmental pollution that involves establishing a list of toxic chemicals presenting a significant risk to or via the environment. These so-called priority pollutants are 126 hazardous substances that include pesticides, hydrocarbons, heavy metals, explosives, chlorinated aliphatics, other chlorinated compounds, phenolics, benzene, toluene, ethylbenzene and xylenes (BTEX). Yet priority pollutants are not the only contributing part of the large chemical pollution puzzle. There is also a diverse group of new, unregulated contaminants that have found their way, and largely spread, into the environment on a worldwide basis and are considered to be 'emergent'. These include illicit drugs, pharmaceutical and personal care products (PPCPs), flame retardants, antibiotics, antiseptics, industrial additives, disinfection by-products and insect repellents (Thomaidis et al., 2012). What is of great concern is the escalating levels of these pollutants and their derivatives which most frequently pollute the environment and threaten the self-regulating capacity of the biosphere so ultimately imposing serious health risks to humans. It is therefore important to restrain or tone down these ever-increasing priority and emerging pollutants by developing newer and more appropriate cost-effective remediation technologies that are realistic, quick and deployable in an extensive array of physical settings.

At present, biological removal of unwanted organic chemicals from soil and water ('bioremediation or green remediation') is considered as an economic, sustainable and eco-friendly remediation technology (Fig. 1). Bio-/phytoremediation employs microorganisms, green plants or their enzymes to treat contaminated substrates for regaining their original natural condition, without transferring any harmful after-effects to the future (Megharaj et al., 2011). In this context, more recently natural polyphenols (secondary metabolites that constitute a large family of ubiquitous and varied aromatic substances, from simple to complex structures) that are present in all green plants and their residues are employed as a suitable bioremedial option to treat contaminated substrates. Among their bioremedial properties, adsorption, metal chelation and more recently, coagulation are probably the three most fully documented ones (Jeon et al., 2009; Smuleac et al., 2011; Stingu et al., 2012). The most significant features of natural polyphenols are that they are non-toxic, easily biodegradable and are present in almost all plant-derived industrial and household wastes that are considered as garbage. Since the remedial utilization of biopolyphenols is emerging, and to date, studies on the use of natural wastes that are rich in polyphenols are very few, there is much scope for future research on this aspect. This review provides a brief overview of (a) abundance and types of natural wastes, (b) polyphenols and their compounds, (c) sources, distribution and the variation in concentration of phenolic compounds in natural wastes, (d) existing remedial applications of natural polyphenols and (e) strategies for the economic utilization of biowaste-derived polyphenols in the clean-up of contaminated environments. The principal focus of this current review is to assist environmentalists in understanding the value of natural polyphenols as a viable resource with scope for exploitation in remedial work.

2. 'Green waste'—values and scope

Green waste comprises food, forestry, garden, agricultural and biological industrial wastes. It is estimated that 140 billion metric tonnes of biomass are generated globally every year from agriculture (Centore et al., 2014). Almost 60% of the total (6273 million tonnes per annum) food production is lost or wasted (Gustavsson et al., 2011). Fallen branches, leaves and flowers of trees, grass clippings, tree and shrub prunings and weeds from forest management and landscaping are left as trash. Husks, pomace, nut or seed shells, straw and residual stocks are generated steadily by agro-industrial activities. One of the burgeoning global problems is the management and minimization of the rapid increase in volume and type of green waste biomass produced. This is because of improper management and accumulation of waste biomass is contributing towards soil, water and air pollution. It is exacerbated by climate change resulting in negative effects on life in our biome. As the accumulation of these wastes is detrimental, concerns are rising and there is a need to develop eco-friendly technologies for the minimization of green wastes. Recently efforts have been made to transform green wastes into products of commercial utility as they are rich in bioactive compounds (vitamins, minerals, amino acids and polyphenols, see Fig. 2) with potentially productive uses (Balasundram et al., 2006). Although there is an emerging trend in the utilization of green wastes, they are still largely under-utilized

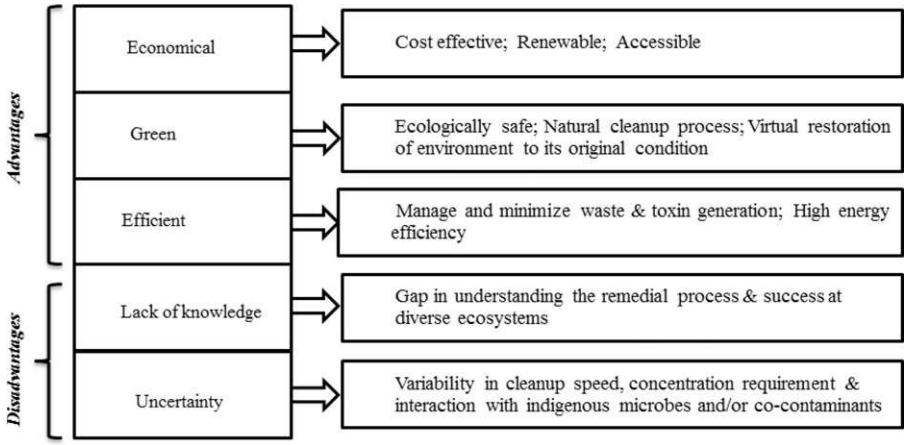

Fig. 1. Advantages and disadvantages of bio-/green remediation.

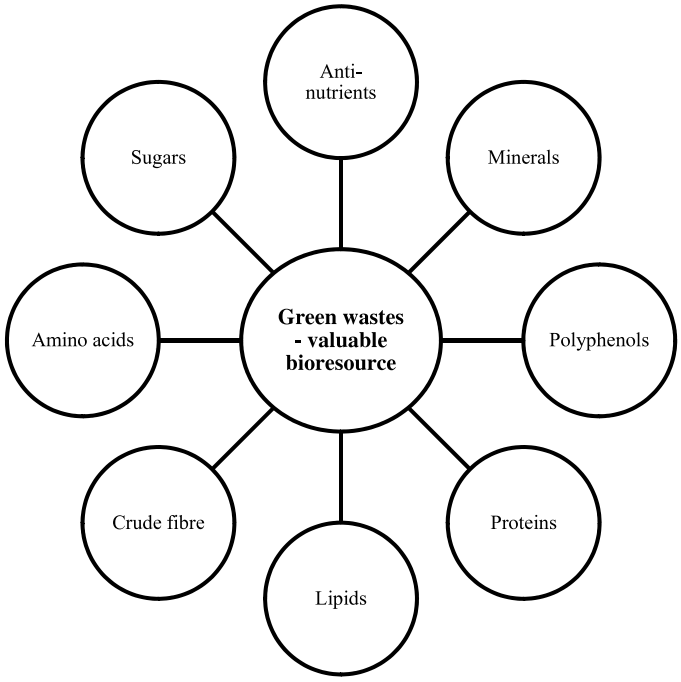

Fig. 2. Valuable bioactive components of green wastes.

and left to rot or are openly burned in the fields or accumulated in landfills, especially in developing countries. Such pollutive practices pose risks to human and ecological health. However, these large volumes of green waste have attractive potentials for large-scale remedial utilization as they are widely available, virtually free and are renewable resources with a multifunctional component, 'polyphenol', that is so far largely unexplored in a bioremedial context.

3. Green waste as renewable polyphenol source—accessibility and concentration disparity

One of the most widely present group of phytochemicals occurring in all vegetative organs of plants and subsequently conserved in dead plant parts identified in green waste is phenols. Plant phenolic compounds account for 40% of organic carbon circulating in the biosphere. Phenolic compounds are defined chemically by the presence of at least one aromatic ring bearing one (phenols) or more (polyphenols) hydroxyl substituents including their functional derivatives (e.g., glycosides and esters) (Hättenschwiler and Vitousek, 2000). Haslam (1996) defined polyphenols as the water-soluble phenolic compounds having molecular mass of 500–4000 dalton and possessing 12–16 phenolic hydroxyl groups and 5–7 aromatic rings per 1000 dalton. In general (poly)phenols can be categorized as low and high molecular weight compounds that include phenolic acids and lignin. The most important class of polyphenol classes are phenolic acids, which include polymeric structures, such as lignans, stilbenes, hydrolyzable tannins and flavonoids. Flavonoids include flavones, isoflavones, flavanones, anthocyanidins pigments responsible for the color of most fruits, flavonols (the most ubiquitous flavonoids in food, for example, quercetin and kaempferol) and flavanols (catechin—monomers and proanthocyanidins—polymers, known as condensed tannins) (Petti and Scully, 2009). These polyphenols are essentially biogenerated in plants through either the shikimate or malonate secondary metabolite pathway or by both. Several thousand plant polyphenols are known (nearly 8000) and have been recognized for their biological properties viz., antioxidant, anticancer, anti-inflammatory effects and in chemical defense mechanisms.

The significant functional property of polyphenols is their ability to scavenge free radicals, chelate metal ions and donate hydrogen atoms or electrons. The structure of polyphenols is a key determinant of their functional activity, followed by their ability to conjugate with other phytochemicals or phenolic compounds, bioavailability, molecular size and stability. The number and position of hydroxyl groups in relation to the carboxyl functional groups of phenolic compounds determine their activity. For example, among phenolic acids, hydroxycinnamic acids possess more functional activity than hydroxybenzoic acids (with –COOH group) due to their CH=CH-COOH group, which ensures greater H-donating ability and radical stabilization (Balasundram et al., 2006). Emerging findings suggest that there are various potential mechanisms of action by which polyphenols may promote bioremediation owing to their metal chelation and radical stabilization activity (Popa et al., 2010). Before understanding the remedial opportunities offered by polyphenols, it is important to understand the source, availability and variations in concentration of polyphenols in natural wastes.

3.1. Food and agro-industrial waste

Phenolic compounds are present in almost all classes of food and agro-industrial residues (fruits, vegetables, oilseeds, nuts, cereals and beverages). Industries and households are the major sources of these residues. (Poly)phenols have so far been identified in several food and agricultural by-products such as rice (*Oryza sativa*) husk (Butsat and Siriamornpun, 2010), peanut (*Arachis hypogaea*) hulls (Win et al., 2011), hazelnut (*Corylus avellana*) shells (Contini et al., 2008), spent coffee (*Coffea arabica*) grounds (Ramalakshmi et al., 2009; Zuorro and Lavecchia, 2012), apple (*Malus* sp.) pomace (Makris et al., 2007; Peschel et al., 2006; Virot et al., 2010; Wijngaard et al., 2009) and several other wastes as illustrated in Table 1. Makris et al. (2007) reported that wine industry by-products, including red grape (*Vitis vinifera*) pomace, seeds and stems are all very rich sources of polyphenols compared to agro-food solid wastes which merit a thorough investigation for their cost-effective exploitation. Gorinstein et al. (2001) found 15% higher total polyphenolic content in peel of citrus fruits (oranges—*Citrus sinensis* and lemons—*Citrus limon*) than those in the peeled fruits. Since, the citrus industry produces large quantities of peel and seed residues, which account for nearly 50% of the total fruit weight with maximum phenols, they can be considered as a potential source of polyphenols. Recently, the by-products of the olive (*Olea europaea*) industry have attracted considerable interest as a source of phenolic compounds, with much attention being focused on olive mill waste which is considered to exceed an annual production of 7 million tonnes (Balasundram et al., 2006). In addition, olive leaves and tree prunings that are other by-products of the olive industry, household wastes have also been explored as a source of polyphenols, although to a lesser extent (Makris et al., 2007; Conde et al., 2009).

The by-products of several other agro-industries have been found to contain higher amounts of polyphenols. Among the agro-food-industrial residues to have been evaluated so far, nutshells and the residues from fruit juice production, particularly peels and pomace, contain relatively high concentrations of total polyphenols in comparison to dried minor crops, waste from canning factories or forest debris (Contini et al., 2008; Peschel et al., 2006; de la Rosa et al., 2010). Grape (*Vitis vinifera*) pomace, pomegranate (*Punica granatum*) peel and pecan (*Carya illinoinensis*) shells contain as high as 54.02 (Makris et al., 2007), 249.4 (Li et al., 2006) and 925 (de la Rosa et al., 2010) mg gallic acid g^{-1} of total phenolics, respectively, and are considered to be the chief green waste resources of polyphenols. Among vegetable residues, red beet (*Beta vulgaris*) pomace was found to contain up to 20.1 mg g^{-1} polyphenols (Peschel et al., 2006). Peels and stalks of the most commonly accumulated household garbage, asparagus (*Asparagus officinalis*), chicory (*Cichorium intybus*), onion (*Allium cepa*), broccoli/cauliflower (*Brassica oleracea* var. *botrytis*) and white cabbage (*Brassica oleracea* var. *capitata*) contain 3.4–37.2 mg g^{-1} dry weight phenolics (Peschel et al., 2006; Wijngaard et al., 2009; Roldán et al., 2008). This amount is at least twice as much contained as that in the edible flesh. Song and Barlow (Soong and Barlow, 2004) evaluated the polyphenolic content of several fruit seeds (jackfruit—*Artocarpus heterophyllus*, mango—*Mangifera indica*, avocado—*Persea americana* and longan—*Dimocarpus longan*) and found that seeds could be a valuable source of polyphenols as they recorded higher phenolic contents than that of the edible flesh. Tomato (*Solanum lycopersicum*) peel has been found to be a rich source of polyphenolic compounds relative to its flesh (Peschel et al., 2006). There are clearly still many opportunities to explore the phenolic profile of other agricultural and horticultural crop residues arising from farms in the form of prunings and cuttings from perennial crops.

It is significant that there exists wide variation in the concentration of total polyphenols among the same and/or different food and agro-industrial wastes reported by several authors as can be seen in Table 1. This variation could be due to a number of intrinsic and extrinsic factors. The intrinsic factors include differences in genus, species, cultivars or organs and extrinsic factors could be any divergent environmental, agronomic, handling and storage practices. Complexity of the phenolic groups (in free or bound form) and use of different analytical methods or standards also have impacts on the under-estimation of natural phenols in source materials. Practical aspects of extraction efficiency have thus to be considered before confirming a residue as potential source of phenolic compounds.

3.2. Forest and garden waste

It is important to stress that of green wastes, forest and garden wastes have not been much explored as a source of phenolic compounds. However, fresh tree leaves have recently been investigated for their polyphenolic content and are widely harnessed for green nanoparticle synthesis because of the stabilization, capping and reducing potentials of tree polyphenols (Machado et al., 2013a). The major polyphenolic constituents of forestry wastes include flavonoids and tannins. Sultana et al. (2007) reported that the bark of neem (*Azadirachta indica*), babul (*Acacia nilotica*) and arjuna (*Terminalia arjuna*) trees contain a total polyphenolic content of 7.8–16.5 mg g^{-1} with 75% being flavonoids. Pine (*Pinus* sp.) seeds are found to be a rich source of polyphenols with the majority (>90%) being tannins (Rakić et al., 2007). Vázquez et al. (2008) observed that eucalyptus (*Eucalyptus* sp.) bark contains 0.11–0.22 mg g^{-1} total phenols, 2.48% lignin, 41.63% cellulose and 62.47% total sugars. A range of 23.7–55.0 mg g^{-1} polyphenols was detected in an aqueous extract of bark from residues of oak (*Quercus* sp.) (Ku et al., 2007). A similar study was made by Pagliosa et al. (2010) who found a total polyphenolic content of 12.5–17.5 mg g^{-1} in the harvest residue (bark) of the yerba mate (*Ilex paraguariensis*) tree. The polyphenolic concentrations in dry residues resulting from cultivation or harvesting activities from trees within and outside of forests, orchards and landscape management (including residential and urban green spaces) are still generally unknown. This is particularly important as the phenolic compounds are ubiquitous in forestry wastes, as that of agro-industrial residues, and if explored further have useful practical implications.

Table 1
Total polyphenolic content of green waste.

Sample	Residue	Total phenols	Reference
(A) Food and agro-industrial waste			
1. *Fruits*			
Apple (*Malus* sp.)	Pomace peel	3.7–33.7[a]; 5.2–9.7[b] 35.2[a]	Makris et al. (2007), Peschel et al. (2006), Virot et al. (2010) and Wijngaard et al. (2009)
Strawberry (*Fragaria* sp.)	Pomace	7.8–17.1[a]	Peschel et al. (2006)
Pear (*Pyrus* sp.)	Pomace	1.0–11.4[a]	Peschel et al. (2006)
Pineapple (*Ananas comosus*)	Pulp, seed and peel	9.1–21.7[a]	de Oliveira et al. (2009) and Kuskoski et al. (2006)
Passion fruit (*Passiflora alata*)	Pulp, seed and peel	41.2[a]	de Oliveira et al. (2009)
Pomegranate (*Punica granatum*)	Peel	249.4[a]	Li et al. (2006)
Grape (*Vitis vinifera*)	Skin and seeds	0.4–9.4[a]	Makris et al. (2007), Katalinić et al. (2010), Lafka et al. (2007) and Selani et al. (2011)
	Pomace	48.2–54.0[a]	
	Seeds	103.3–111[a]	
	Peel	9.7–36.3[a]	
Date palm (*Phoenix dactylifera*)	Seed	0.2–0.4[b]; 35.4[a]	Al-Farsi and Lee (2008) and Ardekani et al. (2010)
Orange (*Citrus sinensis*)	Pomace	9–35[a]	Benelli et al. (2010)
Avocado (*Persea americana*)	Seed and peel	4.3–88.2[a]	Soong and Barlow (2004) and Wang et al. (2010)
Banana (*Musa acuminata*)	Peel	0.01–3.3[a]	Sulaiman et al. (2011)
Mango (*Mangifera indica*)	Kernel	1.8–117[a]	Soong and Barlow (2004) and Soong and Barlow (2006)
Jackfruit (*Artocarpus heterophyllus*)	Seed	27.7[a]	Soong and Barlow (2004)
Tamarind (*Tamarindus indica*)	Seed	94.5[a]	Soong and Barlow (2004)
Acerola (*Malpighia emarginata*)	Pulp and peel	94.6[a]	de Oliveira et al. (2009)
Star fruit (*Averrhoa carambola*)	Pomace	32.2[a]	Shui and Leong (2006)
Rambutan (*Nephelium lappaceum*)	Rind	762[a]	Palanisamy et al. (2008)
Quince fruit (*Cydonia oblonga*)	Peel and seed	0.3–7.1[a]	Silva et al. (2004)
Longan (*Dimocarpus longan*)	Seed	0.2[a]	Soong and Barlow (2006)
2. *Vegetables*			
Red beet (*Beta vulgaris*)	Pomace	0.4–20.1[a]	Peschel et al. (2006)
Tomato (*Solanum lycopersicum*)	Peel	10.6–39.9[a]	Peschel et al. (2006)
Asparagus (*Asparagus officinalis*)	Peel and stalk	4.2–50.1[a]	Peschel et al. (2006)
Chicory (*Cichorium intybus*)	Stem and stalk	2.3–33.5[a]	Peschel et al. (2006)
Onion (*Allium cepa*)	Bagasse	3.3–4.1[b]	Roldán et al. (2008)
	Peel	37.2[a]	
Broccoli (*Brassica oleracea* var. *botrytis*)	Stems	4.94[a]	Wijngaard et al. (2009)
White cabbage/cauliflower (*Brassica oleracea*)	Cut-offs	3.4–4[a]	Wijngaard et al. (2009)
Potato (*Solanum tuberosum*)	Peel	9.7.7[a]	Makris et al. (2007)
3. *Nuts*			
Walnut (*Juglans regia*)	Husk	31.6–33.7[a]	Oliveira et al. (2008)
Hazelnut (*Corylus avellana)*	Shell	56.6–72.2[a]	Contini et al. (2008)
4. *Beverages*			
Coffee (*Coffea* sp.)	Residue	1.4–17.5[a]	Ramalakshmi et al. (2009) and Zuorro and Lavecchia (2012)
5. Oilseeds			
Peanut (*Arachis hypogaea*)	Hull	27.6[a]	Win et al. (2011)
6. *Cereals*			
Rice (*Oryza sativa)*	Husk	1.2–2.2[a]	Butsat and Siriamornpun (2010)
7. *Legumes*			
Cowpea (*Vigna unguiculata*)	Seed	0.08–0.2[a]	Siddhuraju and Becker (2007)
(B) Forestry/tree waste			
Olive (*Olea europaea*)	Leaf	40.2[a]	Makris et al. (2007) and Conde et al. (2009)
	Tree pruning	0.02–0.02[a]	
Bambangan (*Mangifera pajang*)	Peel	22.9[a]	Abu Bakar et al. (2009)
	Kernel	103.3[a]	
Carob (*Ceratonia siliqua*)	Kibbles	13.8[a]	Makris et al. (2007)
Tarap (*Artocarpus odoratissimus*)	Seed	14.7[a]	Abu Bakar et al. (2009)
Eucalyptus (*Eucalyptus globulus*)	Bark	0.1–0.2[a]	Vázquez et al. (2008)
Oak (*Quercus* sp.)	Acorn	0.2[a]	Rakić et al. (2007)
	Bark	23.7–55.0[a]	
Pecan (*Carya illinoinensis*)	Shell	86.4–925[a]	de la Rosa et al. (2010)

[a] mg gallic acid equivalent g^{-1} dry extract.
[b] mg catechin equivalent g^{-1} dry extract.

4. Are polyphenols a bioremedial agent?—the natural way

Though not extensive, studies carried out in recent decades have confirmed the bioremedial role of polyphenols (Fig. 3). They may coagulate and decolorize water contaminated with the synthetic dyes (Jeon et al., 2009), chelate heavy metals and promote *in situ* inactivation of heavy metal ions in waters (Stingu et al., 2012). Moreover, polyphenols participate in nanoparticle synthesis which is useful in remediating a variety of pollutants in aqueous systems (Smuleac et al., 2011). Flavonoids seem to be particularly promising plant

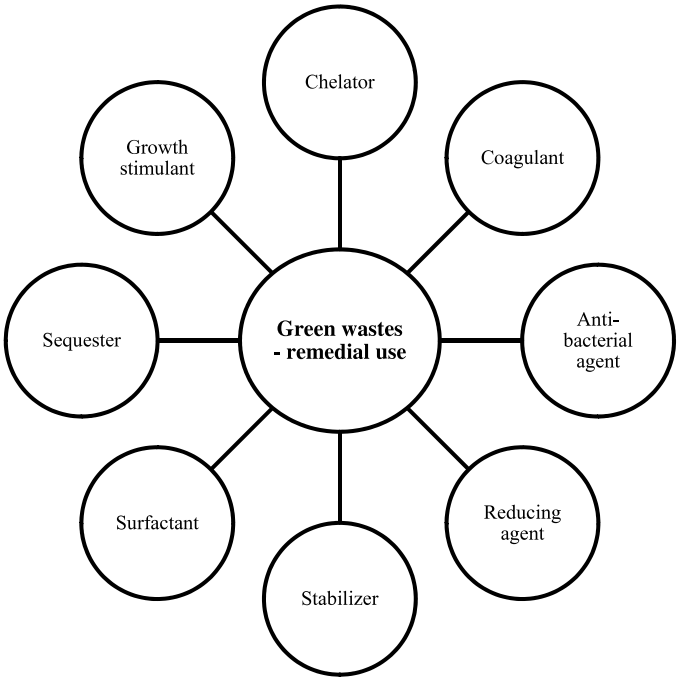

Fig. 3. Bioremedial potential of green wastes.

promoting and defense molecules (Treutter, 2006) which has more implication in phytoremediation. Since polyphenols largely occur in green wastes, they could be used at a reasonable cost in the clean-up of contaminated soil environments.

4.1. Greener generation and sustainable use of nanoparticles in bioremediation

One of the technologies that has emerged as the science of 'all things small' (1–100 nm) and is anticipated to have massive impact in the clean-up of contaminated zones is 'nanoremediation'. Nanoparticles hold promise in addressing challenging site conditions by encouraging remedial success due to their high reactivity and greater number of reactive sites that allow increased contact and rapid reduction of contaminant concentrations (Tratnyek and Johnson, 2006). With rising concerns on the eco- and biological impact of nanostructured noble metals that have found reliable remedial use, the focus on creating non-toxic, 'green' nanomaterials is found attractive and is increasing significantly (Paul et al., 2013). Polyphenol-rich natural extracts are generally chosen for green nanoparticle (NP) synthesis because phenols are non-toxic (biodegradable) and water-soluble at room temperature. Furthermore, polyphenols contribute to greater metal ion reduction and subsequently control the size, morphology, transport and stability of the nanostructures formed (Wang, 2013). In addition, natural polyphenols form nano ions involving a very simple synthesis mechanism that can easily be scaled-up cost-effectively, with greater compatibility for safer environmental applications. The green synthesis of nanoparticles with natural polyphenols involves the following three simple steps: (1) formation of polyphenol complexes with the metals in solution; (2) bioreduction of metal ions to zero-valent particles and (3) final surface capping of the synthesized nanoparticles with oxidized phenolics. The intermittent reduction and final capping by natural polyphenols helps to extend the reactive lifetime of the relatively short-lived nanoparticles in comparison to those that are synthesized chemically (Wang et al., 2014).

Forest, food and agro-industrial products and their residues that are rich in polyphenols are documented as being powerful reducing agents for the production of single or bimetallic gold (Au), silver (Ag), iron (Fe), copper (Cu), platinum (Pt), titanium oxide (TiO_2), carbon (C) and palladium (Pd) NPs as outlined in Table 2. For example, polyphenolic constituents such as gallic acid, epicatechin, rutin and ellagic acid present in acacia (*Acacia nilotica*) pod extracts were shown to be responsible for the reduction and protection of Ag^+ ions and AgNPs (20–30 nm in size with distorted spherical shapes), and had greater efficacy in catalyzing the reduction of benzyl chloride (Jebakumar Immanuel Edison and Sethuraman, 2013). Wang et al. (2014) successfully prepared FeNPs via a simple one-step eucalyptus leaf polyphenol extract and demonstrated its great potential for the *in situ* remediation of swine wastewater. Wang demonstrated that the natural polyphenols play a crucial role in protecting the nanoparticles from oxidation and aggregation and improved their dispersion by acting as a capping agent. AgNPs (size 20 nm) synthesized from haritaki (*Terminalia chebula*) fruit extracts attained an efficient catalytic reduction of the basic dye, methylene blue (Edison and Sethuraman, 2012). In yet another study (Wang, 2013), eucalyptus-based FeNPs were found to possess excellent dye (acid black 194) removal capacity with potential for water purification and groundwater remediation through adsorption. The maximum adsorption capacity was 1.6 g acid black 194 per gram of FeNPs at 25 °C.

Catalytic hydrogenation of nitroaromatic pollutants such as 2-nitrophenol and 2-nitroaniline was observed by Dauthal and Mukhopadhyay (2013) using flame tree (*Delonix regia*) leaf extract based PdNPs. The water soluble polyphenolic compounds (phenolic acids = 1.28 g of gallic acid per 100 g fresh weight of leaf, and flavonoids = 0.83 g of quercetin per 100 g fresh weight of leaf) in leaf extract of *D. regia* was observed to serve as the key electron donor and played significant role in biosynthesis of PdNPs. According to the authors, the mechanism of polyphenol mediated PdNP synthesis comprised the following steps. Initially, the two hydroxyl groups present in the benzene ring of phenolic acids (gallic acid) participated in the bioreduction of metal ($PdCl_2$) ions. Since phenolic compounds have the capability to donate electrons and easily oxidize into its quinones, $PdCl_2$ first oxidized gallic acid and formed an intermediate Pd complex. Subsequently, Pd^{2+} ions formed with concomitant oxidation of gallic acid. The Pd^{2+} ion then reduced to Pd^0 in the presence of

Table 2
Nanomaterials synthesized from polyphenol-rich natural sources for health and environmental use.

Nanoparticle	Naturals	Extract
Ag^0	Coffee (*Coffea arabica*)	Residue
	Tea (*Camellia sinensis*)	Residue
	Magnolia (*Magnolia kobus*)	Leaf
	Golden shower tree (*Cassia fistula*)	Leaf
	Amla (*Emblica officinalis*)	Fruit
	Sorghum (*Sorghum bicolor*)	Bran
	Hibiscus (*Hibiscus rosa sinensis*)	Leaf
	Betel (*Piper betel*)	Leaf
	Grape (*Vitis vinifera*)	Pomace
	Neem (*Azadirachta indica*)	Leaf
	Ashoka tree (*Saraca asoca*)	Leaf
	Java plum (*Syzygium cumini*)	Leaf
	Babul (*Acacia nilotica*)	Pod
	Cashew (*Anacardium occidentale*)	Leaf
	Dwarf mulberry (*Morus nigra*)	Leaf
	Chebulic myrobalan (*Terminalia chebula*)	Leaf
	Nerium (*Nerium oleander*)	Leaf
	Banana (*Musa paradisiaca*)	Peel
	Sugar apple (*Annona squamosa*)	Peel
	Orange (*Citrus sinensis*)	Peel
	Mandrin (*Citrus unshiu*)	Peel
Au^0	Tamarind (*Tamarindus indica*)	Leaf
	Leadwood tree (*Terminalia catappa*)	Leaf
	Coleus (*Coleus amboinicus*)	Leaf
	Stevia (*Stevia rebaudiana*)	Leaf
	Safflower (*Carthamus tinctorius*)	Dry flower
	Camphor tree (*Cinnamomum camphora*)	Fruit
	Zinger (*Zingiber officinale*)	Leaf
	Jack fruit (*Artocarpus heterophyllus*)	Leaf
	Common fig (*Ficus carica*)	Pod
	Plum (*Prunus domestica*)	Fruit
	Ironwood tree (*Memecylon umbellatum*)	Leaf
	Pomegranate (*Punica granatum*)	Peel
	Citrus (*Citrus limon, C. reticulata, C. sinensis*)	Fruit
Fe^0	Tea (*Camellia sinensis*)	Leaf, spent waste
	Eucalyptus (*Eucalyptus* sp.)	Leaf
	Oak (*Quercus* sp.)	Leaf
	Pomegranate (*Punica granatum*)	Leaf
	Guava (*Psidium* sp.)	Fruit
	Orange (*Citrus sinensis*)	Peel pith
Pd^0	Grape (*Vitis vinifera*)	Pomace
	Royal Poinciana (*Delonix regia*)	Leaf
Pt^0	Gum kondagogu (*Cochlospermum gossypium*)	Leaf
Zn^0	Alfalfa (*Medicago sativa*)	Residue
TiO_2^0	Sugar apple (*Annona squamosa*)	Peel
C^0	Pomelo (*Citrus maxima*)	Peel

free electrons which were produced during bioreduction reaction. Finally, collisions of the neighboring Pd^0 atoms lead to the formation of palladium nanoparticles.

Machado et al. (2013a) flagged antioxidant properties of the phenolic compounds as promising reducing agents for nano-sized zero-valent iron (nZVI) production. Machado and his colleagues evaluated the viability of 26 different tree leaves that had high polyphenol contents as well as elevated antioxidant capacities to produce extracts that are capable of reducing Fe(III) to form nZVI. The results showed that dried leaves of oak (*Quercus* sp.), pomegranate (*Punica granatum*) and green tea (*Camellia sinensis*) produce extracts with higher antioxidant activities (oak = 90 mmol L^{-1}; pomegranate = 70 mmol L^{-1}; green tea = 50 mmol L^{-1}) and polyphenolic contents (oak = 0.6 mmol L^{-1}; pomegranate = 1.1 mmol L^{-1}; green tea = 1.5 mmol L^{-1}) and are viable to form nZVI. In yet another study of Machado et al. (2013b), nZVI produced from polyphenol-rich natural extracts (grape pomace/leaves—*Vitis vinifera* and black tea—*Camellia sinensis*) were able to remediate soil contaminated with a common anti-inflammatory drug, ibuprofen (remediation efficiency >95%) through the complementation of the process with a catalyzed nZVI Fenton-like reaction. Fenton's reaction is a process mediated by iron catalyzed hydrogen peroxide referred as Fenton's solution that is used to oxidize contaminants.

Recently, Liang et al. (2014) reported the reduction of Cr(VI) using recyclable lead and palladium NPs immobilized on procyanidin/grated eggshell membrane. In this, procyanidin, a natural polyphenol with abundant phenolic hydroxyls, provided stable binding sites for chelating the metal precursors and aided the reduction of toxic Cr(VI) to the less toxic Cr(III) ions. Thus polyphenol-rich sources are not only involved in the direct synthesis of nanoparticles but can also be used as a component in the fabrication of novel nanoparticles to enhance their remedial efficiency. It is evident that remedial uses of green nanoparticles are still at the bench-scale level, and require more attention in future research for large or field-scale application. Their remedial applications are currently restricted to dyes and metals, and to the best of our knowledge, studies are not yet reported on their potential for removing the majority of persistent organic pollutants (POPs) and emerging pollutants of concern (EPC) which are of raising concern and thus require much more focus. Detailed investigation of green nanoparticle synthesis from the commonly available and polyphenol-rich household and industrial wastes can be given more importance with use as bioremedial agents.

4.2. Role in phytoextraction: 'sequestrants' to form chelates with heavy metals

One of the proven but still emerging, cost-effective and eco-friendly green remediation technologies for environmental clean-up is phytoremediation. Phytoextraction is one type of phytoremediation approaches and is best suited for the remediation of diffusely polluted areas, where pollutants occur in a relatively low concentration. Phytoextraction relies on the use of plants to extract metals from soil and translocate them to shoots. The resulted plant biomass can then be harvested thereby removing the heavy metals from the site (Stingu et al., 2012). Recently polyphenols have been proposed to be used as biodegradable chelates to enhance the metal soil availability and accumulation in plants while avoiding leaching risks. Promising metal (Zn, Ni and Cd) solubilization potential of polyphenols has been demonstrated for gallic acid when applied at a dose of 10–20 mM kg^{-1} when compared with other natural bioactive compounds (citric, oxalic and vanillic acid) (Nascimento, 2006). Ignat et al. (2011) and Popa et al. (2010) suggested the use of aqueous polyphenolic extracts obtained from diverse resources, viz., red grape (*Vitis vinifera*) seed and spruce (*Picea abies*) wood bark as 'sequestrants' to form chelates with heavy metals or as heavy metal biosolubilizers. In this context, Stingu et al. (2012) evaluated the use of natural polyphenolic extracts as natural amendments in Cd(II) phytoextraction and obtained enhanced bioaccumulation in the case of oat (*Avena sativa*) by use of polyphenol-containing aqueous extracts from spruce (*Picea abies*) bark, chesnut (*Castanea sativa*) shells and milkweed (*Asclepia syriaca*) that contained 0.08–0.23 mg g^{-1} flavonoids and 0.16–0.17 mg g^{-1} tannins. Despite the significant role of polyphenols in phytoextraction, other studies have not been reported elsewhere and have scope for exploration. It is important to note that the composition and concentration of polyphenols derived from raw materials affect their potential to chelate toxic metals and thereby reduce risks. Hence, it is necessary to investigate the suitability and standardize the concentrations of polyphenols derived from different sources to enhance phytoremediation of metal-contaminated soil zones. Furthermore, it will be important to understand the effects of polyphenols on plant metabolism and/or enzyme activity and the role of polyphenols in contributing to the defense mechanisms of plants under conditions of stress.

4.3. Biosorption: coagulation, complexation and reduction

Multiple technologies are employed to achieve the regulatory standards for treated water discharge such as sorption, coagulation/flocculation, chemical oxidation, membrane separation, electrochemical, aerobic and anaerobic microbial degradation. Among all the methods in current use, adsorption is much preferred due to its cheapness and effectiveness. More recently, however, the use of biosorbents for wastewater treatment is found to be promising owing to their relative abundance, ecofriendly and green nature. Due to continuous search for new, easily available biosorbents with maximum remedial potential, natural polyphenols have lately been identified as profitable biosorbents and flocculants for heavy metals and dyes in water treatment (Copello et al., 2013). Polyphenols are advantageous when used as biosorbents, being sparingly soluble in water and immobilization avoids leaching (Anirudhan et al., 2012). At the same time, polyphenolic immobilization provides sorbent mechanical stability and a physical support for water remediation, which also allows their reuse. In fact, natural polyphenols can act as ligands and attach with inorganics, e.g., iron or chromium. Furthermore, polyphenols have excellent ability to form non-covalent interactions with (in)organic materials (Jeon et al., 2009).

4.3.1. Natural organic coagulants for dye removal from the aqueous ecosystem

One of the most efficient methodologies for soluble organic dye removal is to move the dyes to a separable solid phase from the dissolved state. This technique is based upon the coagulation of soluble agents whereby flocs formed with coagulants can be treated by means of a mechanical process. Of late and in order to guarantee environmental and health safety, the use of natural organic coagulants (NOCs) has been advocated as a sustainable technology for wastewater treatment. Anionic hydroxyphenyl groups (polyphenols) in NOCs are reported to be the main organic ingredients causing dye removal by gradual floc formation (Jeon et al., 2009; Sánchez-Martín et al., 2010). Jeon et al. (2009) demonstrated cationic dye (Malachite Green and Crystal Violet) precipitation concomitant with strong decolorization, resulting from gradual coagulation of the dye by grape seed-derived natural polyphenols, catechin and tannic acid. The initial non-covalent interactions between the cationic dyes and naturally derived polyphenols, attributed to electrostatic interaction between cationic dye and hydroxyphenyl groups, further progressed into stronger aggregates (flocs), which is essential for dye removal from water. The toxicity of dye-contaminated water was also found to be effectively reduced by natural polyphenol treatment. Recently, tannins (vegetal water-soluble polyphenolic compounds with molecular weights between 500 to several thousand daltons) are presented as a promising source of coagulants for dye removal. For example, in a pilot study (Sánchez-Martín et al., 2010), a new tannin-based flocculant—'Tanfloc' (derived from acacia—*Acacia mearnsii*, modified by a physico-chemical process) amended at a dose of 92.2 mg L^{-1}, removed 95% of an acid dye from textile industry wastewater. In yet another study, two tannin-based coagulants derived from acacia—*A. mearnsii* (Acquapol S5 T) and quebracho—*Schinopsis balansae* ('Silvafloc') showed high affinity and eliminated Alizarin Violet 3R from textile effluents (Beltrán-Heredia et al., 2009). Beltrán-Heredia et al. (2010) showed 80% Palatine Fast Black WAN, 75% Acid Red 88, 50% Alizarin Violet 3R, 20% Carmine Indigo, 20% Chicago Sky Blue 6B and 10% Eriochrome Cyanine R removal (dye concentration 200 mg L^{-1}) from aqueous solution using 'Tanfloc'. Future studies should focus on the large-scale use of NOCs in the bioremediation of real wastewater samples.

4.3.2. Biological metal sorbents in liquid media

Polyphenolic compounds present in natural materials are most important sorption sites for the binding of metal ions. Thus biosorption (selective sequestering of metal-soluble species that result in the immobilization of metals by biomass) of metal ions occurs as a result of ion exchange or complex formation between metal ions and the functional hydroxyl groups of polyphenols on the surface of the biomaterial. Metal sequestration by natural polyphenols can be also by chelation, reduction or a coupled mechanism (Tsezos et al., 2006). In the last decade, researchers have synthesized adsorbents from commercial tannins and then used them to remove various metal ions from wastewater. Yurtsever and Şengil (2009) highlighted the metal removal efficiency of several tannin biosorbents and showed that Pb(II) sorption by a modified quebracho (condensed tannin that has a polymeric structure containing flavonoid units—a combination of resorcinol, catechol and pyrogallol) tannin resin had a maximum adsorption capacity of 86.2 mg g^{-1} at pH 5 and 290 K.

Immobilization of polyphenols (tannic acid) may contribute to enhancement in adsorption efficiency of metals (Liu et al., 2010). A combined ion-exchange complex formation and surface adsorption/complexation process could result in such enhancements, as shown by Üçer et al. (2006), who used tannic acid-immobilized activated carbon for the removal of Cu(II), Cd(II), Zn(II), Mn(II) and Fe(III) ions from aqueous system. Using yerba mate (*Ilex paraguariensis*) milling residual dust as the polyphenol source, Copello et al. (2013) developed a low-cost biosorbent hybrid material, whereby yerba mate waste polyphenols were immobilized within a SiO_2 matrix to form an interpenetrated polymer after glutaraldehyde cross-linking. The hybrid material exhibited an adsorption efficiency of 1.78 mg g^{-1} for Cr(VI), 2.7 mg g^{-1} for Pb(II) and 4.5 mg g^{-1} for Cr(III). Chand and Pakade (2013) reported that polyphenols are highly efficient for the removal of Pb(II) than for other metals since Pb ions were observed to possess a particularly strong binding capacity with polyphenols which enhanced their removal by the surface adsorption method. Chand and Pakade proved this concept using apple pomace that showed Pb(II) adsorption of 16.39 mg g^{-1} (optimum contact time 80 min; adsorbent dose 0.8 g in 50 mL solution, pH 4). Chemically-modified gambir (*Uncaria gambir*) powder that contained polyphenolic compounds, flavonol monomers, catechin and epicatechin was capable of adsorbing 9.9 mg g^{-1} Cu(II) ions at 333 K (initial metal ion concentration: 10 mg L^{-1}, pH 5, adsorbent dose 0.3 g in 50 mL solution). The potential of biomass rich in polyphenolic groups to bind and remove heavy metals from aqueous solutions has been shown by several other authors. For example, Cd(II) and Pb(II) removal by sawdust of *Pinus sylvestris* (Taty-Costodes et al., 2003), Cr(VI) removal by coffee polyphenol–formaldehyde/acetaldehyde resin (Mulani et al., 2013), Cu(II), Zn(II), Cd(II) and Ni(II) removal by pelletized ponderosa pine (*Pinus ponderosa*) bark (Oh and Tshabalala, 2007) and Cu(II) biosorption by biomass derived from Algerian Sahara plants (Cheriti et al., 2011).

The existing studies thus illustrate that it is possible to remove metal ions from aqueous solutions with polyphenol-rich adsorbents. However, there remains a question if these adsorbents are suited for the removal of a wider class of environmental pollutants other than heavy metals. In the case of metal removal using polyphenolic substrates, it is necessary to understand the mechanisms involved as there are more chances for coupled reactions, viz., reduction or oxidation with the adsorption process. So far this aspect is not completely explored. Reduction of toxic Cr(VI) to the 100-fold less toxic Cr(III) by spent tea and coffee dusts rich in polyphenolic compounds (which include catechins, flavones, anthocyanin and phenolic acids) was shown by Prabhakaran et al. (2009). Cr(VI) adsorbed onto a material can be further reduced to Cr(III) through the polyphenol aromatic ring which is rich in electrons. Natural polyphenols thus act as an electron donation component which ultimately results in the detoxification and removal of Cr(VI) from water. This was elegantly shown by Huang et al. (2013) using mangosteen *(Garcinia mangostana)* peel.

5. Future research needs—towards 'greener' generation

Remedial utilization of natural polyphenols is not complete. The use of polyphenols in the remedial sector is currently restricted to their biosorption and coagulation mechanisms. However, there are wider prospects recommending future research using polyphenol-rich natural wastes (as we have listed in Table 3) that might pave the way for 'greener' generation where simultaneous economic minimization and management of wastes followed by pollutant remediation can be attained hand-in-hand, if the beneficial properties of polyphenols are thoroughly understood, studied extensively and then harnessed efficiently.

5.1. Can natural polyphenols be used as amendments at long-term contaminated sites?—the bioaugmentation and/or biostimulation perspective

One promising possibility is to use polyphenol-rich green wastes as organic amendments either as single or as combinations for the bioremediation of historically contaminated sites i.e., to aid biostimulation. It is because polyphenols are degradable and when degraded can be utilized as a carbon source, which may result in the improvement of growth of the indigenous soil bacterial community under polluted conditions. Moreover, some polyphenol-rich green wastes are also sources of biosurfactants, e.g., citrus peel—a rich source of polyphenols and rhamnolipid biosurfactants (George and Jayachandran, 2009) which can act as a pollutant bioavailability enhancer and increase pollutant accessibility to the indigenous microbes. They may also chelate the toxic heavy metal fractions. Furthermore, polyphenols can support soil nutrient dynamics. In essence, polyphenols can bind to sesquioxides and prevent phosphate sorption and thus contribute to the maintenance of P availability for microbial utilization. Polyphenols can also retain exchangeable inorganic cations (K, Ca and Mg) by providing high sorption sites and can maintain the availability of metal micronutrients (e.g., Mn, Fe) by the formation of organic complexes (Hättenschwiler and Vitousek, 2000). These properties of polyphenols can enhance the field-scale bioremediation processes when used as amendments. However, it is necessary to optimize the amendment concentration (as polyphenols) in order to obtain a positive response in the indigenous microflora. Unfortunately, there is no direct evidence as to what extent natural polyphenols can increase or decrease nutrient retention in problematic soils and this aspect should be considered as a research priority.

It is doubtful if polyphenols can mimic organic pollutants with similar chemical structure (benzene rings), e.g., polyaromatic hydrocarbons (PAHs), and hence if polyphenols can be harnessed as a component in any microbial inoculum developed in order to conserve the PAH degrading genes in the long run. In this context, it is necessary to study the combined effects of isolated microbial strains and polyphenols on the bioremediation of pollutants. The potential of polyphenols on maintaining the survival and functional ability of the isolated strains should also be studied which altogether can bring a new insight into the prospects for bioaugmentation.

5.2. Application in microbe-assisted phytoremediation

The positive feedbacks of polyphenols in conserving nutrients and creating a more favorable medium for root growth are as follows: minimizing N losses; complexing Al, Mn and Fe, thereby reducing potential Al toxicity and P-fixation in soil; regulating organic matter dynamics, leading to the accumulation of organic matter with cation exchange capacity to minimize leaching of nutrient cations (Northup et al., 1998); their potential to act as a plant growth regulator and as heavy metal biosolubilizer/chelator (Stingu et al., 2012); their ability to support the growth of some pollutant-degrading microorganism by acting as a nutrient source and enhance the pollutant metabolism by inducing the pollutant degrading enzymes (Singer et al., 2003), for example, PCB metabolism as shown by Donnelly et al. (1994). These

Table 3
Future research needs in natural polyphenol-based bioremedial approaches: towards green transformation for eco-innovation.

Research area	Existing knowledge	Future research scope
Characterization and screening of natural polyphenol rich wastes		
Interaction between natural polyphenols and priority pollutants		
Prospective of natural polyphenols to remove emerging contaminants of concern		
Mechanism of nutrient cycling at contaminated zones with respect to aging and at different ecological conditions		
Potential to enhance contaminant bioavailability		
Economics of waste and toxin minimization by use as amendments during bioremediation		
Effects of natural polyphenols on indigenous soil microbes—growth and/or activity inhibitors or regulators		
Potential as growth stimulators during phyto/rhizoremediation		
Nanofertilizer production		
Bacteriostat in drinking and wastewater treatment		
Carriers in commercial bioremedial inoculum production		
Economic green replacers of synthetics		
Harnessing the redox potential of natural polyphenols to enhance the efficacy of existing remedial options		
Use of crude polyphenol extracts as natural organics in pollutant removal		
Exploitation for the large-scale remediation of real contaminated sites		

The following symbols represent the existing level of knowledge and scopes for future research:
 —No (0%); —Low (≤25%); —Average (≤50%); —Reasonable (≤75%); —Absolute (100%).

collectively highlight the superiority of polyphenols for utilization in microbe-assisted phytoremediation. The application of polyphenol-rich green wastes as suitable substrates or as nutrient additives during microbe-assisted phytoremediation may enhance the growth and activity of the phytoremediating plant plus microbes, and ultimately accelerate the rate of bioremediation due to the combined effects of the above listed benefits. The role of individual phenolic compounds in assisting rhizoremediation or phytoextraction can be studied individually.

5.3. Scope for nanofertilizer production

It is our understanding that the future 'blue sky' scope for natural polyphenols exploitation is not only limited to the synthesis of green nanoparticles, but they may also find application in developing nanofertilizers. In this, polyphenol-rich green wastes could be pooled and reduced to nanosize by adopting simple techniques such as ball milling and can then be used as a slow-release fertilizer for application in long-term contaminated environments. To assist the bioremediation of pollutants, the nanosized polyphenolic source could be modified with surfactants in order to accelerate the potential of the developed nanofertilizer to remove the expected charged pollutants. In some cases, pollutant-degrading strains can be amended with the developed nanofertilizer for promising effects. This will pave the way for the development of a new, green and economical commercial product for use by the remedial sector.

5.4. Hitching antibacterial properties against pathogens—biofilters in wastewater treatment

The antimicrobial activity of natural polyphenols against pathogens has been studied extensively (Coppo and Marchese, 2014). This property coupled with the traits to chelate heavy metals or coagulate dyes in wastewater can be used in the development of a bed of polyphenol-rich green waste as a biofilter to treat heavy metal- or dye-contaminated water. In this case, along with the immobilization and removal of pollutants, the pathogenic coliforms would also be eliminated, thereby making the treated water safer for utility. This approach could replace the existing commercial activated carbon-based filters and result in a greener, cost-effective and sustainable approach, as natural polyphenols are widely available and are easily biodegradable after utilization. As polyphenols are capable of scavenging free radicals, the risks of developing diseases, particularly cancer, are relatively low (Sahpazidou et al., 2014) when polyphenol-treated water is distributed for human utility in comparison to existing filtering media that are used to treat wastewater. Indeed, fabrication of polyphenol-based nanofilters can also be considered on a commercial basis in order to treat industrial wastewater. Furthermore, the potential of crude natural polyphenolic extracts to act as bacteriostats requires testing. If successful, a powdered form of pure polyphenols extracted from natural waste materials could be marketed and added at a standardized concentration to inhibit or completely retard the proliferation of specific genera of pathogenic bacteria.

6. Conclusion

Considerable research on natural polyphenols now provides the necessary body of knowledge to understand their biotechnological potentials. With existing information on the unique traits of natural polyphenols, environmentalists have to look for possible bioremedial roles for polyphenols. Existing research integrating natural polyphenols and the priority for bioremediation of emerging organic and inorganic pollutants is limited. There exists possibility in the future for the development of biopolyphenol-based sustainable and marketable nanofertilizers, biofilters, bacteriostats, natural additives and amendments for use in the treatment of contaminated soil and water. Characterization or genetic coding of individual polyphenolic compounds, e.g., flavonoids, flavonol, tannin, etc., is needed in order to explore their biotechnological potential on a reliable basis. The use of genetic coding will be valuable to define the specific functional role of individual phenolic compounds for more direct application and would also help to identify the source of interesting phenolic compounds. What is now more important is to integrate the pollutant-degrading microbes and polyphenol-rich natural waste materials and carefully study their function and responses in pollutant degradation. It is not the case that all natural polyphenols aid in the minimization of risks due to the persistence of pollutants. Only future research will provide sufficient knowledge on the remedial potentials offered by natural polyphenols. However, green waste rich in polyphenols is no more to be considered as a waste but as a resource with scope for multifunctional remediation. It will be in our hands to develop the use of different food, agro-industrial and tree waste materials that are the notable sources of polyphenols in the successful removal of environmental pollutants.

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