Ethical field research, phytochemical studies and importance of alien species but near threatened plant *Hyphaene dichotoma* in Union territories of Diu and Gir district, Gujarat, India

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Abstract: The present study emphasis on alien species '*Hyphaene dichotoma'* found in districts of Gir, Gujarat, India. It is one of the most important useful plants in the world. All parts of *Hyphaene dichotoma* palm have a useful role such as fiber and leaflets which is used to weave baskets and *Hyphaene dichotoma* nuts which have antioxidants and secondary metabolites such as tannins, phenols, saponin, steroids, glycosides, flavonoid, terpenes and terpinoids. Also, roots, stems and leaves are used in medicine, ropes and baskets. Studies on anti-inflammatory, antioxidant, antimicrobial, anticancer and pharmacological potential of *Hyphaene dichotoma* extracts and its major phytoconstituents like the phenolic, essential oil and flavonoid compounds are extensively studied. The result of Antioxidant property shows that the fruit of *Hyphaene dichotoma* is a source of potent antioxidants.

Index terms: *Hyphaene dichotoma,* phytochemistry, antioxidant property.

I. INTRODUCTION

Hyphaene dichotoma (White) Furtado commonly known as Indian *Hyphaene dichotoma* palm has been categorized as Near Threatened by the IUCN in 2014. The tree is nick named as 'Hoka' tree. The species was introduced in India by Portuguese sailors from Africa and was planted in the coastal region of Gujarat. This species is declining in numbers due to anthropogenic factors like continued deforestation, change in land use patterns, unscrupulous extraction of wild sources, etc. These factors pose barriers for seed germination. Field and laboratory research is critical for developing a fuller understanding of the biodiversity richness. The tree tends to grow close to groundwater but can also grow farther away. It is also found in parts where water occurs, in oases and wadis, and is widely distributed near rivers and streams, sometimes on rocky slopes. It does not do well in waterlogged areas; it is very resistant to bush fires. Trees occur on silty soils on river and stream banks and on rocky hilly slopes throughout the study area. The tree is nowadays recklessly cut down and anthropogenic influence has made its distribution to nearly threaten. The aim of the study is to protect the tree from its endangering status by reflecting and exploring the phytochemical and medicinal values.

II. MATERIAL AND METHOD

2.1. *Hyphaene dichotoma* fruit extract

Fresh mesocarp of *Hyphaene dichotoma* collected were ground into fine powder. Ten grams of *Hyphaene dichotoma* fruit powder were put into a glass bottle, containing 600 ml boiling deionized water, with continuous stirring for 30 min. The fruit extract was filtered by Whatman number 1 filter paper, using a Buchner funnel and water suction. The clear filtrate was evaporated to dryness under vacuum using the Rota-Vapor at 35–40°C, and further dried by freeze–drying. The yield was 2.39 g (23.9% by weight). The freeze–dried fruit extract was redissolved in methanol:water (1:1, v/v) to 50 mg/ml concentration and aliquots were kept at 20°C until used. Sample extract was warmed to redissolve precipitated materials prior to use.

2.2. Phytochemical Analysis of *Hyphaene dichotoma* Mesocarp Extract

Phytochemical screening for tannins, anthraquinones, flavonoids and carbohydrates was carried out using the method described by Trease and Evans (1989; 1997); while glycosides, alkaloids, reducing sugars, monosaccharides, ketones, pentoses and terpenes by Sofowora (1982), Odebiyi and Sofowora (1978) and saponins by Harborne (1973).

2.2.1. Test for Tannins (Ferric chloride test)

Two millilitre of the crude solution of the extract was added to few drops of 10% Ferric chloride solution (light yellow). The occurrence of blackish blue color shows the presence of garlic tannins and a greenblackish color indicates presence of catechol tannins.

2.2.2. Test for saponins (Frothing Test)

Three millilitres (3ml) of the crude solution of the extract was mixed with 10ml of distilled water in a test-tube. The test-tube was stoppered and shaken vigorously for about 5 minutes; it was allowed to stand for 30 minutes and observed for honeycomb froth, which is indicative of the presence of saponins.

2.2.3. Test for Alkaloids

One gram (1g) of the extract was dissolved in 5ml of 10% ammonia solution and extracted with fifteen millilitre of chloroform. The chloroform portion was evaporated to dryness and the resultant residue dissolved in 15 ml of dilute sulphuric acid. One quarter of the solution was used for the general alkaloid test while the remaining solution was used for specific tests.

2.2.4. Mayer's reagent (or Bertrand's Reagent)

Drops of Mayer's reagent was added to a portion of the acidic solution in a test tube and observed for an opalescence or yellowish precipitate indicative of the presence of alkaloids.

2.2.5. Dragendorff's reagent

Two millilitres (2ml) of acidic solution in the second test-tube was neutralized with 10% ammonia solution. Dragendorff's reagent was added and turbidity or precipitate was observed which was indicative of presence of alkaloids.

2.2.6. Tests for Carbohydrate (Molisch's test)

Few drops of Molischs solution was added to 2ml of aqueous solution of the extract, thereafter a small volume of concentrated sulphuric acid was allowed to run down the side of the test tube to form a layer without shaking. The interface was observed for a purple colour, which is indicative of positive for carbohydrates.

2.2.7. Tests for Carbohydrate (Barfoed's test)

One millilitre of aqueous solution of the extract and 1ml of Barfoed's reagent were added into a test-tube, heated in a water bath for about 2 minutes. Red precipitate shows the presence of monosaccharide.

2.2.8. Standard test for Combined reducing sugars

One millilitre of the crude solution of the extract was hydrolyzed by boiling with 5 ml of dilute hydrochloric acid. This was neutralized with sodium hydroxide solution. The Fehling's test was repeated as indicated above and the tube was observed for brick-red precipitate that indicates the presence of combine reducing sugars.

2.2.9. Standard test for Free reducing Sugar (Fehling's test)

Two millilitre of the crude aqueous solution of the extract in a test tube was added 5ml mixture of equal volumes of Fehling's solutions I and II and boiled in a water bath for about 2 minutes. The brick-red precipitate indicates the presence of reducing sugar.

2.2.10. Test for Ketone

Two millilitre of crude aqueous solution of the extract was added a few crystals of resorcinol and an equal volume of concentrated hydrochloric acid, and then heated over a spirit lamp flame and observed for a rose colouration, that shows presence of ketone.

2.2.11. Test for Pentoses

Two millilitre of the aqueous solution of the extract was added an equal volume of concentrated hydrochloric acid containing little phloroglucinol. This is heated over a spirit lamp flame and observed for red colouration, indicative of presence of pentoses.

2.2.12. Test for Phlobatannins (Hydrochloric acid test)

Two millilitre of the crude aqueous solution of the extract was added dilute hydrochloric acid and observed for red precipitate that indicates presence of Phlobatannins.

2.2.13. Test for Cardiac glycosides

Two millilitre of the crude aqueous solution of the extract was added 3 drops of strong solution of lead acetate. This was mixed thoroughly and filtered. The filtrate was shaken with 5ml of chloroform in a separating funnel. The chloroform layer was evaporated to dryness in a small evaporating dish. The residue was dissolved in a glacial acetic acid containing a trace of ferric chloride; this was transferred to the surface of 2ml concentrated sulphuric acid in a test tube. The upper layer and interface of the two layers were observed for bluish-green and reddish-brown colouration respectively, which indicates the presence of cardiac glycosides.

2.2.14. Test for Steroids (Liebermann-Burchard's test)

The amount of 0.5g of the crude aqueous extract was dissolve in 10ml anhydrous chloroform and filtered. The solution was divided into two equal portions for the following tests. The first portion of the solution above was mixed with one ml of acetic anhydride followed by the addition of 1ml of concentrated sulphuric acid down the side of the test tube to form a layer underneath. The test tube was observed for green colouration indicative of steroids.

2.2.15. Test for Steroids (Salkowski's test)

The second portion of solution above was mixed with concentrated sulphuric acid carefully so that the acid formed a lower layer and the interface was observed for a reddish-brown colour indicative of steroid ring.

2.2.16. Test for Flavonoids (Shibita's reaction test)

One gram (1g) of the crude aqueous extract was dissolved in methanol (50%, 1- 2ml) by heating, then metal magnesium and 5-6 drops of concentrated hydrochloric acid were added. The solution when red is indicative of flavonols and orange for flavones.

2.2.17. Test for Flavonoids (Pew's test)

To five millilitre (5ml) of the crude solution of the water extract was added 0.1g of metallic zinc and 8ml of concentrated sulphuric acid. The reaction mixture was observed for red color indicative of flavonols.

2.2.18. Test for Anthraquinones (Borntrager's reaction for free anthraquinones)

One gram (1g) of the powdered fruit was placed in a dry test tube and 20ml of chloroform was added. This was heated in steam bath for five minutes. The extract was filtered while hot and allowed to cool. To the filtrate was added equal volume of 10% ammonia solution. This was shaken and the upper aqueous layer was observed for bright pink colouration, which is an indication of the presence of Anthraquinones. Control test were done by adding 10ml of 10% ammonia solution in 5ml chloroform in a test tube.

2.3. Evaluation of antioxidant activity

For spectroscopic quantitative determination of antioxidant activity, the reaction time for each activity assay was selected, such that maximum formation or disappearance of a particular chromogen occurred at a fixed concentration of test material. All the antioxidant activity assayed, except for reducing power, was measured as decrease in absorbance. Scavenging effects (%) or chelating effects (%) were calculated from the absorbance data using the equation

$$\text{Scavenging or Chelating Effect (\%)} = \left(1 - \frac{A_{Sample}{}^{\lambda Activity\ Assay}}{A_{Control}{}^{\lambda Activity\ Assay}}\right) X\ 100 \quad \text{.....(3.2)}$$

Where A is absorbance and λ Activity Assay the wavelength of absorbance measurement for the particular assay.

Four replicate reactions were performed for each concentration point and the mean ± 1 S.D percentage scavenging or chelating effect values were plotted against concentration of test material in mg per ml reaction volume. The reactions in these assays were concentration- dependent and were allowed to reach saturation (maximum inhibition in assay) to accurately determine EC50 (amount of test material by mass required to produce 50% maximum inhibition per ml reaction volume) by fitting data into a non-linear regression algorithm (GraphPad Prism, version 4). The reducing power assay measures increase in

absorbance in the presence of a large excess of oxidized substrate (Fe^{3+}). Four replicate absorbance values were calculated as means ± 1 S.D and were also plotted against concentration of test material in mg per ml reaction volume. The reducing power, $RP_{0.5AU}$ (defined as amount of material in l g or mg per ml reaction volume that produces 0.5 absorbance unit at λ_{700nm}), was obtained from the plot of the absorbance data (mean ± 1 S.D.) fitted into a straight line by linear regression method.

2.3.1. Hydrogen-donating activity

Hydrogen-donating activity was measured by direct hydrogen donation to DPPH radical, as described by Yamaguchi, Matoba, and Junji (1998). The disappearance of the free radical can be tracked by absorbance measurement at λ_{517nm}. The reaction mixture (1.10 ml) contained 100 µl of test *Hyphaene dichotoma* extract and ascorbic acid (0–0.02 mg) and 1.00 ml of DPPH solution (0.1 mM in methanol). The control contained all the reaction reagents except test material. The reaction mixture was shaken well and allowed to react for 20 min at room temperature. The remaining DPPH free radical was determined by absorbance measurement at λ_{517nm} against methanol blanks. The percentage scavenging effect was calculated from the decreased in absorbance against control (without added test material).

2.3.2. Superoxide radical-scavenging activity

Superoxide radical-scavenging activity was determined by absorbance measurement of the blackish blue formazan product of superoxide addition to nitro blue tetrazolium (NBT) substrate, according to the method of Nishikimi, Rao, and Yagi (1972). Superoxide was generated chemically by the reduction of phenazine methosulphate (PMS), using b-NADH as the electron donor in the presence of dissolved molecular oxygen in the reaction solution. The reaction mixture (1.00 ml) contained 700 µl of test gallic acid (0– 0.5 mg) in methanol, 100 µl of b-NADH (1 mM in water), 100 µl of NBT (1 mM) in 1 M-phosphate buffer, pH 7.8, and 100 µl of PMS (120 µM in water) added in that order and the mixture allowed to react at RT for 10 min. The control contained all the reaction reagents except the test material. The reaction was terminated by adding 40 µl of concentrated HCl (10M) and absorbance measured at λ_{560nm} against blanks that contained all components except test material and PMS. The percentage scavenging effect was calculated from the decrease in absorbance against control (without added test material).

2.3.3. Reducing power

Reducing power was measured by direct electron donation in the reduction of $Fe^{3+}(CN^-)_6$ to $Fe^{2+}(CN^-)_6$ as described by Yen and Chen (1995). The product was visualized by the addition of free Fe^{3+} ions after the reduction reaction, by forming the intense Prussian blue colour complex, $Fe_4{}^{3+}[Fe^{2+}(CN^-)_6]3$, and quantitated by absorbance measurement at λ_{700nm}. The reaction mixture (1.16 ml) contained 160 µl of test *Hyphaene dichotoma* extract and ascorbic acid (0–32 µg), 500 µl of potassium ferricyanide $K_3Fe^{3+}(CN^-)_6$, (1% w/w in water) and 500 µl of 0.2 M phosphate buffer, pH 6.6. The control contained all the reaction reagents except the test material. The mixture was incubated at 50°C for 20 min and was terminated by addition of 500 µl of 10% (w/v) TCA, followed by centrifugation at 3000 rpm for 10 min. Five hundred microliter of the supernatant upper layer was mixed with 500 µl water and 100 µl ferric chloride ($Fe^{3+}Cl_3$, 0.1% w/v in water) and the absorbance was measured at λ_{700nm} against blanks that contained distilled water and phosphate buffer. Increased absorbance of the reaction mixture indicates increased reducing power of sample.

2.3.4. Inhibition of substrate site-specific hydroxyl radical formation

Inhibition of substrate site-specific hydroxyl radical formation was determined by direct absorbance measurement of the formation of TBARS using 2-deoxy-Dribose as substrate and site of hydroxyl radical formation as described by Aruoma (1994). The procedure was the same as that described for measuring non-site specific hydroxyl radical-scavenging activity (above) except that EDTA was left out of the reaction mixture. The percentage scavenging effect was calculated from the decreased in absorbance against control (without added test material).

2.3.5. Fe^{2+}-chelating activity

Iron (II)-chelating activity was measured by inhibition of the formation of iron (II)-ferrozine complex after preincubation of the reaction mixture with test material according to the method of Decker and Welch (1990). The reaction mixture (1.50 ml) contained 500 µl test *Hyphaene dichotoma* extract, Na2EDTA (0–0.07 µg), 100 µl FeCl2 (0.6 mM) and 900 µl methanol. The control contained all the reaction reagents except test material. The mixture was shaken well and left at RT for 10 min; 100 µl of ferrozine (5 mM in

methanol) were then added, mixed and left for another 5 min to complex the residual Fe^{2+}. The absorbance of the Fe^{2+}–ferrozine complex was measured at λ_{562nm} against methanol blanks. The percentage chelating effect was calculated from the decreased in absorbance against control (without added test material).

2.3.6. Hydroxyl radical-scavenging activity

Hydroxyl radical-scavenging activity was determined by measuring the formation of thiobarbituric acid-reactive substances (TBARS) using 2-deoxy-D-ribose as substrate, as described by Aruoma (1994). An EDTA–Fe^{2+} complex was employed to generate hydroxyl radical in situ by the decomposition of H_2O_2, using ascorbic acid as the reducing agent for EDTA–Fe^{3+}, to initiate the decomposition reaction (non-site specific method). Because the reaction is incompatible with methanol, the latter was removed from the *Hyphaene dichotoma* fruit by multiple rotary evaporations under vacuum and each test sample redissolved in water at a lower concentration for solubility before use. The reaction mixture (1.00 ml) contained 250 µl of test material (*Hyphaene dichotoma* extract [0–12.5 mg], or gallic acid [0–1.25 mg]), 250 µl of potassium phosphate buffer, pH7.4 (100 mM), 100 µl of 2-deoxy-D-ribose (28 mM), 200 µl of Fe^{3+}–EDTA (a mixture of 100 lM $FeCl_3$ + 104 µM Na_2EDTA in 1:1 ratio, v/v), 100 µl of H_2O_2 (1 mM) and 100 µl of ascorbic acid (1 mM). H_2O_2 and ascorbic acid were added last in that order to initiate the generation of hydroxyl radical. The control contained all the reaction reagents except test material. The reaction mixture was mixed by vortex and then incubated for 1 h at 37°C. To terminate the reaction and form TBARS chromogen, 50 µl of butylated hydroxyanisole (2%, w/v in methanol), 1 ml of trichloroacetic acid (2.8%, w/v in water) and 1 ml of 2-thiobarbituric acid (1%, w/v in water) were added, tubes capped and the mixture heated in a boiling bath for 20 min. The reaction was stopped by a short incubation period in an ice water bath. 2.0 ml of 1-butanol were then added and the tubes mixed before centrifugation at 3000 rpm for 5 min to clarify the 1-butanol phase for absorbance measurement at λ_{532nm} against 1-butanol blanks. The percentage scavenging effect was calculated from the decrease in absorbance against control (without added test material).

III. RESULTS AND DISCUSSION

3.1. Morphology of tree

Hyphaene is pleonanthic, dioecious and includes species with small to massive and tall, solitary or basally clustered, erect or creeping stems (Figs. 1) costapalmate leaves, often persistent when old; and conspicuously polymorphic, brown to orange fruits Stem usually dichotomously branched, leaves costa-palmate, divided into numerous segments, segments divided for more than half their length. Petiole spiny, usually with black upwardly. curved hooks. Hastula often oblique. Dioecious pleonanthic. Inflorescence intra-foliar and branched. Flowers small, male and female equal in size. Male flower: sepals 3; petals 3, united to form a stalk; stamens 6, filaments free, anthers ovate, bifid at base, fixed by their back. Female flower: sepals and petals 3, staminode rudimentary; stigmas 3, sessile. Ovary 3-celled, usually only one developing into fruit giving fruit with basal stigma remains; sometimes more than 1 ovule develops, ill-defined pore in apical position opposite the embryo. Drupe oblong, sweet; albumen bony, homogeneous, hollow, embryo at tip vertical.

Figure 1: ***Hyphaene dichotoma*** **(a) Tree; (b) Fruit (c) Dry powder of fruit**

Stem dichotomously branched, 8-10 m long; trunk, dark-black, annulated, sometimes covered with persistent leaf scars, smooth; bark grey-ash coloured in lower half, green on upper part. Leaves about 1 m broad, flabellate, multifid, long petiolate. Inflorescences branched about 6, bearing about 6 flowering branchlets, digitately arranged the next bearing 5, and the apical branch which is convex and only 8 mm in diameter bearing only 2. The branches arise from their respective spathes. Fruits pretty, regularly obovate pyriform.

3.2. Phytochemical analysis

The extracts of *Hyphaene dichotoma* was evaluated for phytochemical analysis. The quantitative phytochemical screening revealed the presence of low level of tannins, steroids and moderate level of saponins, carbohydrates, cardiac glycosides, flavonoids, Terpenes and Terpinoids. The phytochemical estimations was done using following tests.

TABLE 1: Quantitative phytochemical analysis of the crude extract of *Hyphaene dichotoma*

Phytochemical constituents	Test	Inference
Tannins	Ferric chloride	+
	Formaldehyde	+
	Chlorogenic acid	+
Saponins	Frothing	++
Alkaloids	Dragendorff's	-
	Mayer's	-
	Wagner's	-
Carbohydrates	Molisch's	+
	Barfoed's	-
	Combine reducing sugar	++
	Free reducing sugar	++
	Ketone's	+
	Pentoses	++
Phlobatannins	Hydrochloric acid	-
Cardiac glycosides	General test	++
Steroids	Lieberma's	+
	Salkowski's	+
Flavonoids	Shinoda's	++
	Ferric chloride	+
Terpenes/ Terpinoids	Liebermann-Buchard's	++
	Salkowski's	++
Anthraquinones	Free anthraquinones	-

Key: - Absent, + Low, ++ Moderate

The quantitative phytochemical screening of *Hyphaene dichotoma* revealed the presence of low level of tannins, steroids and moderate level of saponins, carbohydrates, cardiac glycosides, flavonoids, Terpenes and Terpinoids. Extracts of *Hyphaene dichotoma* are used in the treatment of hypertension, bilharzias and as a hematinic agent. (Burkill HM, 1997). It reduces hyperlipidemia in nephrotic syndrome and leads to decrease in risk of glomerulosclerosis and atherosclerosis and consequently the nature, safe and nontoxic *Hyphaene dichotoma* fruit could be of great merit for use as hypolipidemic drugs. It is also a good hypocholesterolemic agent and possesses anti-imflamatory action.

3.3. Antioxidant property

As the plant showed a significantly good antioxidant property, it has valuable importance in medicine. Traditionally the tree is categorized as medicinal plant owing to its richness value in healing majority of diseases. Free radicals can react with biomolecules, causing extensive damage to DNA, protein, and lipid, which are considered to be related to aging, degenerative diseases of sging, cancer (Oberly TD 2002; EL-Beltagi HS and Mohamed HI. 2013). Antioxidants play an important role in the later stages of cancer development. The oxidative stress is defined as the imbalance between oxidants and antioxidants in favor of the oxidants potentially leading to damage in human cells. They play a role in preventing the formation of free radicals, which are responsible for the harmful oxidative processes (EL-Beltagi HS et al. 2013).

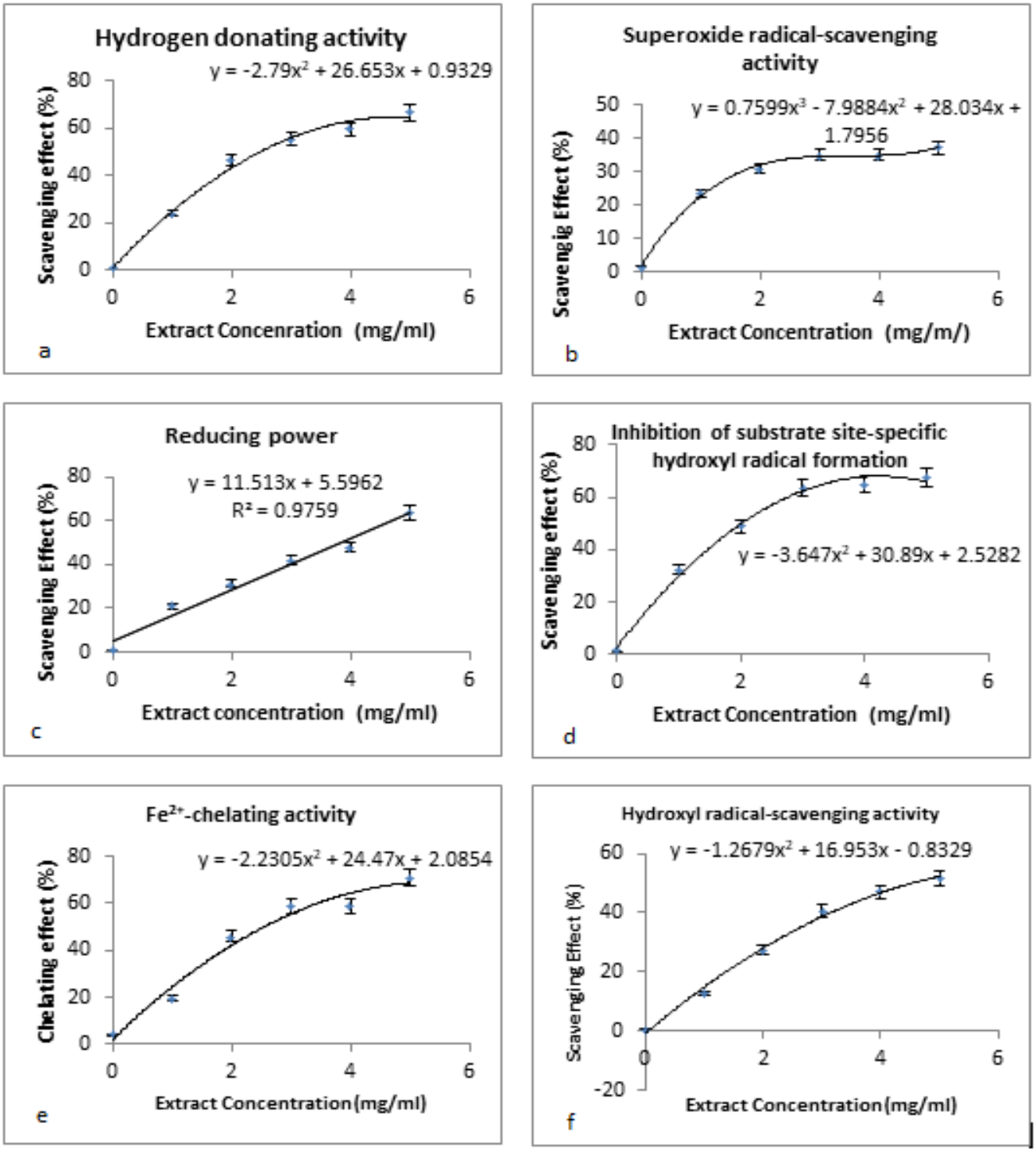

Figure 2: (a) Hydrogen-donating activities; (b) Superoxide radical-scavenging activities; (c) Reducing power; (d) Inhibition of substrate site-specific hydroxyl radical formation; (e) Fe2+-chelating activities; (f) Hydroxyl radical-scavenging activities of *Hyphaene dichotoma* tree. The absorbance values were converted to scavenging effects (%) and data plotted as the means of replicate scavenging effects (%) ± 1 S.D (n = 4) against extract concentration in weight extract per ml reaction volume. The data were fitted into a nonlinear regression algorithm.

TABLE 2: EC_{50} and $RP_{0.5AU}$ values

Antioxidant activities	*Hyphaene dichotoma* extracts	Ascorbic acid EC_{50}	Na_2EDTA	Gallic acid
Hydrogen-donating activity	201(165)[a] 4(3)[b]	2[c]	-	-

Superoxide radical-scavenging activity	3821(124)[a] 59(1.67)[b]	-	-	124[c]
Reducing power	943(111)[a] 20(2.07)[b]	$RP_{0.5\ AU}$ 8[c]	-	-
Inhibition of substrate site-specific hydroxyl radical formation	573(133)[a] 8(3.65)[b]	-	-	3[c]
Fe^{2+}-chelating activity	420(115)[a] 6(2.06)[b]	-	4[c]	-
Hydroxyl radical-scavenging activity	383(3.14)[a] 2(0.01)[b]	-	-	1112[c]

The EC50 and RP0.5AU values for *Hyphaene dichotoma* fruit were calculated from data presented in Figs. 2(a–f). The values for the pure compounds (ascorbic acid, EDTA and gallic acid) were calculated from data obtained from similar experiments and scavenging effect (%), chelating effect (%) or reducing power (absorbance) versus test material plots (data not shown). Values shown are in µg extract[a], lgGAE[b] of extract or µg pure compound[c] per ml reaction volume. All values were rounded to the nearest lg. Values in () are relative to pure compound for the particular assay. –Not determined.

TABLE 3: Antioxidant activities of *Hyphaene dichotoma* fruit extracts

	Name of Activity test	Value
1	Hydrogen-donating activity	0.03[a] 2.85[b]
2	Superoxide radical-scavenging activity	0.04[a] 1.49[b]
3	Reducing power	0.03[a] 3.24[b]
4	Inhibition of substrate site-specific hydroxyl radical formation	0.02[a] 3.987[b]
5	Fe^{2+}-chelating activity	0.05[a] 1.34[b]
6	Hydroxyl radical-scavenging activity	1.03[a] 164[b]

The EC_{50} and $RP^{3}_{0.5}$ AU values for *Hyphaene dichotoma* fruit from Table 2 were converted to (1 and 3) mmol ascorbic acid equivalent, (5) mmol EDTA equivalent and (2,4,6) mmol gallic acid equivalent per g extracts or per gGAEb of extract.

3.3.1. Hydrogen donating activity

The role of an antioxidant is to remove free radicals. One mechanism through which this is achieved is by donating hydrogen to a free radical in its reduction to an unreactive species. Addition of hydrogen would remove the odd electron feature which is responsible for radical reactivity. The hydrogen-donating activity, measured using DPPH radical as hydrogen acceptor, showed that the fruit extract contained 0.03 mmol ascorbic acid equivalent/g extract of activity.

3.3.2. Superoxide radical-scavenging activity

Superoxide radical can be generated in vitro and assayed by the PMS/b-NADH/NTB system. Again, an antioxidant activity can come about by antioxidant donation of hydrogen or electron to superoxide or by direct reaction with it. Using this assay, the fruit was found to contain 0.04 mmol gallic acid equivalents/g extract.

3.3.3. Reducing power

An electron-donating reducing agent contributes to antioxidant activity by its capacity to donate an electron to free radicals, which results in neutralization of the reactivity of the radical, and the reduced species subsequently acquires a proton from solution. Another reaction pathway in electron donation is the reduction of an oxidized antioxidant molecule to regenerate the ‘‘active’’ reduced antioxidant. Using an assay to directly measure transfer of electrons to Fe^{3+}, the fruit was found to contain 0.03 mmol ascorbic acid equivalents/g extract of activity.

3.3.4. Inhibition of substrate site-specific hydroxyl radical formation

Due to the high reactivity of hydroxyl radical, it was recognized that measurement based on scavenging hydroxyl radical, such as the non-site specific method above, does not relate to antioxidant protection of an antioxidant molecule in vivo (Halliwell et al., 1995). This is because the radical is more likely to be scavenged by direct reaction with other surrounding molecules before it can attack its target molecule. Using this inhibition assay, the fruit was found to contain 0.02 mmol gallic acid equivalents/g extract of activity.

3.3.5. Ferrous ion-chelating activity

Iron and copper are essential transition metal elements in the human body for the activity of a large range of enzymes and for some proteins involved in cellular respiration, O2 transport and redox reactions. But, because they are transition metals, they contain one or more unpaired electrons that enable them to participate in one-electron transfer reactions. Hence, they are powerful catalysts of autoxidation reactions, such as participation in the conversion of H2O2 to $OH^{\cdot}$ in the Fenton reaction and in the decomposition of alkyl peroxides to the highly reactive alkoxyl and hydroxyl radicals (Lloyd, Hanna, & Mason, 1997). Due to this property, transition metal chelation to form low redox potential complexes is an important antioxidant property (Halliwell, Aeschbach, Loliger, & Aruoma, 1995) and measuring chelation of iron(II) is one method for assessing this property. Since the reaction is dependent on the affinity of an antioxidant towards iron (II) in relation to ferrozine, the assay is affected by both binding constant and concentration of antioxidant and thus only strong iron antioxidant chelator is detected. With this assay, the fruit was found to contain iron (II)-chelating activity 0.05 mmol EDTA equivalent/g extract.

3.3.6. Hydroxyl radical-scavenging activity

An antioxidant ability to scavenge hydroxyl radical is an important antioxidant activity because of the very high reactivity of hydroxyl radical that enables the radical to react with a wide range of molecules found in living cells, such as sugars, amino acids, lipids and nucleotides. Hydroxyl radical can be generated in situ by decomposition of hydrogen peroxide by high redox potential EDTA–Fe2+ complex (non-site specific) and, in the presence of 2-deoxy-D-ribose substrate, it forms TBARS which can be measured (Aruoma, 1994). Antioxidant activity is detected by decreased TBARS formation, which can come about by antioxidant donation of hydrogen or electron to the radical or by direct reaction with it. With this assay the fruit was found to contain 1.03 mmol gallic acid equivalents/g extract in hydroxyl radical-scavenging activity.

IV. CONCLUSION

Hyphaene dichotoma, a well-known plant for its antioxidant, and bioactive activities is an important valuable tree found within the districts of Gir near Una and Union territories of Diu. This tree is an alien species but now threatened for its biodiversity richness due to anthropogenic influence. The tree is also planted and conserved for its valuable importance however its existence is near to endanger despite of Government actions. Traditional importance and local lucrative assets of the tree should be taken into mind and thence the conservation and maintenance should be born in mind as a step for this valuable threatening species.

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