Season, Solvent Type and Concentration Modulate in Vitro Antioxidant and Nitric Oxide Radical Scavenging Capabilities of Fignut (*Jatropha Gossypfolia*) Extract

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Abstract

The influence of season , solvent type and concentration on the phenolic and flavonoid contents including in vitro antioxidant and nitric oxide radical scavenging activities of stem bark and leaf extracts of fignut (Jatropha gossypfolia) were investigated. Season , extraction solvent type and concentration were critical determinants of the total phenolic and flavonoid contents including in vitro antioxidant and nitric oxide radical scavenging activities of the plant parts. The stem bark extract expressed potent in vitro nitric oxide radical scavenging capabilities in dry season than wet season , which was significant (P< 0.05) , with water, 70% acetone , and absolute(100%) acetone as extraction solvents. The order of the in vitro nitric oxide radical scavenging activities of the stem bark extracts in dry season was: aqueous(76.59%) > 70% acetone(62.96%)> absolute acetone(59.20%).Water was the most promising solvent for in vitro nitric oxide radical scavenging capability for the stem bark in dry season . The aqueous stem bark could be exploited for the treatment of inflammatory disorder because of its potent in vitro nitric oxide scavenging activity. The total phenolic contents in aqueous extracts were 24.40±0.11(leaf, dry season), 14.20±0.45(stem bark , dry season), 5.76±0.09 (Leaf, wet season) and 1.70 ± 0.05 mg/ml(stem bark , wet season).In addition,70% acetone was the best candidate extraction solvent for total phenolics extraction of the leaf in dry season(39.60±0.53mg/ml).Also, 70% acetone was the most promising extraction solvent for in vitro antioxidant activity assay and displayed the most remarkable in vitro antioxidant potential in leaf extract of the plant in wet season(123.90±1.52% activity)The in vitro antioxidant activity of the leaf extracts in 70% acetone and absolute acetone were slightly higher in wet season than dry season , but the difference was not significant(P>0.05).The leaf extracts was a dull scavenger of nitric oxide in vitro during the dry , but expressed potent antioxidant activity in the same season. In conclusion, there was a clear evidence that the in vitro antioxidant and nitric oxide radical scavenging activities of the stem bark extracts of J.gossypifolia were significantly higher in all the three solvents in dry season (P<0.05) than wet season.

Key words: bioactivity, antioxidant, oxidant, phytomedicine and pro-oxidant

Introduction

Nitric oxide is involved in inflammation (Moncada and Higgs, 1991).*Jatropha gossypifolia* is a gregarious shrub with palmately lobed leaves , and possessed dark red , crimson or purplish flowers(Das and Das , 1994). The common English name for J. gossypifolia is fignut (Odebiyi and Sofowora , 1998). J.gossypifolia belongs to the family Euphobiaceae(Misra and Misra, 2010).The plant has been used ethnomedically for the treatment of cough ,tuberculosis , bacterial infections and cancerous growth(Aiyelagbe *et al.,* 2007).The stem latex of the plant is used as haemostatic agent and its mechanism of action as haemostatic agent found to be by precipitation of coagulation factors(Oduola *et al.*, 2005a,b).The stem latex of J.gossypfolia is routinely used by local and some urban dwellers in Southern Nigeria to stop bleeding from nose, gum and injured skin (Oduola *et al.*, 2007).

In 2005, Western Australia banned Jatropha gossypifolia as invasive and highly toxic to people and animals (Misra and Misra, 2010).The stem bark extract of the plant showed a potent anti-inflammatory activity (Purohit and Reena,2011).

The plant possessed cyclic peptide called cyclogossine B (Auvin –Guette *et al.,* 1997). Jatrophenone , a diterpene with antibacterial activity is present in the plant(Ravindranath *et al.,* 2003).The latex of the plant contained cyclic octapeptides (cyclogossine A and B) (Horsten *et al.,* 1996).The aerial part of the plant contained gossypiline (a new lignan)(Das *et al.,* 1998).The plant contains alkaloid jatrophine in the root and bark , and a lignin , jatrodien is found in its stem (Matsuse *et al.,* 1999; Omoregbe *et al.,* 1996).

To the best of our knowledge , there is no in vitro comparative research work on the influence of season , and solvent type (absolute acetone, 70% acetone and distilled water) and on in vitro antioxidant and nitric oxide scavenging potentials of Jatropha gossypifolia .Therefore, this research was designed to investigate the influence of season , solvent type and concentration on the total phenolic and flavonoid content including antioxidant and nitric oxide radical scavenging activities of the stem bark and leaf extracts of Jatropha gossypifolia in vitro.

Materials and Methods

Collection of plant material

The plant parts(stem and leaves) were collected from Oke-Anu , Ogbomoso, Nigeria on 11th July , 2011 at about 9.25am , during the raining season ; while the second batch was collected on 6th February 2012 around 11.30am , during the dry season.

Preparation of plant extracts

Two grammes (2g) of each plant part was soaked for 1 hr in 40ml of each solvent. Distilled water , 70% acetone and absolute acetone were used as extraction solvents. The solution was filtered using whatman filter paper.The filterate obtained was used for the analysis of parameters of interest.

In Vitro Analyses

In vitro nitric oxide radical scavenging potential assay

The in vitro nitric oxide scavenging activity was estimated according to the method of Marcocci *et al(* 1994).To 1ml sample, 1ml of sodium nitroprusside (10mM, aqueous) and 1 ml buffer (sodium phosphate buffer,0.2M) were added. The mixture was incubated at room temperature for 150 mins (2hr 30 min) followed by the addition of 0.1ml Griess reagent. The absorbance of the pink colour solution was read at 540nm on a spectrophotometer. The pink chromophore generated during diazotization of nitrite ions with sulphanilamide and subsequent coupling with N-naphthyl ethylene diamine dihydrochloride was measured spectrophotometrically at 540nm.

The in vitro NO scavenging activity of the sample was calculated by using the following formula:

Nitric oxide scavenging activity (%) = (Acontrol - A sample)/ Acontrol x 100
Where Acontrol = The absorbance of the control (reaction mixture in the absence of sample).
Asample = The absorbance of the reaction mixture.

In vitro antioxidant activity (DPPH based) assay

The in vitro antioxidant activity of the sample was quantitated according to the traditional method of Blois(1958).To 1ml of plant extract, 1ml of methanolic solution of 2,2-diphenyl -1-picryl –hydrazyl (DPPH)(0.2mM) was added.The mixture was incubated in the dark for 30min.The absorbance of the yellow colour solution was read at 517nm on a spectrophotometer using distilled water as blank.

$$\text{DPPH scavenged (\%)} = \frac{(A_{DPPH} - A_{sample})}{A_{DPPH}} \times 100$$

Total phenol content assay

The phenolic content of the sample was determined according to the method of Taga *et al* (1974).To 0.1ml of sample, 2ml of sodium carbonate solution (0.2% w/v) was added , followed by the addition of 0.1ml of Folin-

Ciocalteu reagent(10% , v/v).The mixture was incubated for 10 min. The absorbance of the blue colour solution was read at 480nm .The concentration of total phenolic (mg/ml) in the extract was extrapolated from pyrocatechol calibration curve.

Total flavonoid content assay

The flavonoid content of the sample was determined according to the method of Lamaison and Carnet(1990).To 0.5ml sample , 0.5ml of 70% $AlCl_3.6H_20$ (2%) was added and the mixture incubated for 10min . The absobance of the yellow colour solution was read at 430nm after 10min on a spectrophotometer using distilled water as blank. The total flavonoid concentration (mg/ml) of the extract was obtained from a calibration curve using quercetin as a standard flavonoid.

Statistical Analysis

Student's t-test was used for statistical analysis .P value less than or equal to 0.05 or 0.001 were considered significant.

Results

Table 1: The trend of in vitro antioxidant activity, nitric oxide scavenging potential , total phenolics and flavonoid contents of Jatropha gossypifolia aqueous extract during the dry and wet season

PARAMETER	TREND OF IN VITRO BIOACTIVITIES AND SELECTED PHYTO CONSTITUENTS ANALYSES
	Decreasing order of bioactivity during wet and dry season using water as extraction solvent
Antioxidant activity(%)	Leaf (dry season) > Stem bark(dry season) > Leaf(wet season) > Stem bark (wet season)
Nitric oxide radical scavenging activity(%)	Stem bark (dry season)>Leaf(wet season)> stem bark(wet season) > Leaf (dry season).
Total flavonoid(mg/ml)	Stem bark (dry season)> leaf(wet season) > leaf (dry season)> Stem bark (wet season)
Total phenolics(mg/ml)	Leaf(dry season) > stem bark (dry season)> leaf (wet season) > stem bark (wet season)

Using water as an extraction solvent , the leaf extract of J.gossypfolia displayed the highest antioxidant activity (**106.12 %**) during the the season(**Table 1 and Table 5a**). The order of in vitro antioxidant activity of the aqueous extract of the plant during dry and wet season was as presented above(**Table 1**).

The aqueous stem bark extract of the plant exhibited the maximum in vitro nitric oxide radical scavenging (**76.59 %)** during the dry season compared to other parts of the plant. **(Table 1 and Table 5a**).The total flavonoid and phenolics contents displayed maximum value in the stem bark and leaf during the dry season , respectively.

Table 2:The trend of in vitro antioxidant activity, nitric oxide scavenging potential , total phenolics and flavonoid contents of Jatropha gossypifolia(70% acetone extract) during the dry and wet season.

PARAMETER	TREND OF IN VITRO BIOACTIVITY AND SELECTED PHYTO CONSTITUENTS ANALYSES
	Decreasing order of bioactivity during wet and dry season using 70% acetone as extraction solvent
Antioxidant activity (%)	Leaf(wet season) > stem bark (dry season) > leaf (dry season) > stem bark(wet season).
Nitric oxide radical scavenging activity (%)	Stem bark (dry season) >Stem bark (wet season)>Leaf(dry season) > Leaf(wet season).
Total flavonoid(mg/ml)	Leaf dry season > Leaf (wet season) > Stem bark(dry season) > stem bark(wet season)
Total phenolics(mg/ml)	Leaf (dry season) > Stem bark(dry season)>Leaf(wet season) > Stem bark(wet season)

Using 70% acetone as an extraction solvent , the order of antioxidant activity , total flavonoid and phenolics in the stem bark and leaf of the plant was as shown above(**Table 2**).The leaf extract showed the maxixum in vitro antioxidant activity during the wet season when compared to other parts of the plant.The maximum total flavonoid and phenolics contents were observed in the leaf during the dry season.

Table 3:The trend of in vitro antioxidant activity, nitric oxide scavenging potential , total phenolics and flavonoid contents of Jatropha gossypifolia (absolute acetone extract) during the dry and wet season

PARAMETER	TREND OF IN VITRO BIOACTIVITY AND SELECTED PHYTO CONSTITUENTS ANALYSES
	Decreasing order of bioactivity during wet and dry season using absolute acetone as extraction solvent
Antioxidant activity (%)	Leaf(wet season) > Stem bark(dry season) > Leaf(dry season) > Stem bark(wet season).
Nitric oxide radical scavenging activity(%)	Stem bark(dry season) > stem bark(wetseason)> Leaf(dry season) > Leaf(wet season).
Total flavonoid(mg/ml)	Leaf (dry season) > Leaf (wet season) >Stem bark (dry season) > stem bark (wet season)
Total phenolics(mg/ml)	Leaf(dry season) >Stem bark(dry season) >Leaf(wet season) >Stem bark (wet season)

Using absolute acetone as an extraction solvent, the maximum antioxidant and nitric oxide scavenging activities were observed in the leaf during wet season , and the stem bark during the dry season , respectively.The maximum total flavonoid and phenolics contents were obtained in the absolute leaf extracts during the dry season(Table 3).The order of selected in vitro bioactivities , total flavonoid and phenolics was as presented above(Table 3).

Table 4a : The overall trend of in vitro antioxidant and nitric oxide scavenging activities of Jatropha gossypifolia extracts in all selected solvents during the dry and wet season

PARAMETER	THE OVERALL TREND OF SELECTED IN VITRO BIOACTIVITIES
	Decreasing order of bioactivity during wet and dry season using absolute acetone as extraction solvent
Antioxidant activity(%)	Leaf (70% acetone, wet season) > Leaf (absolute acetone , wet season) > Stem bark(absolute acetone, dry season) >Stem bark (70% acetone ,dry season)> Leaf (absolute acetone , dry season) > Leaf (70% acetone , dry season) > Leaf (water, dry season > Stem bark (water, dry season) > stem bark (absolute acetone , wet season) >Leaf (water, wet season) > stem bark (70% acetone, wet season) > stem bark (absolute acetone , wet season).
Nitric oxide radical scavenging activity(%)	Stem bark(water, dry season) > stem bark (70% acetone , dry season) > stem bark (absolute acetone , dry season) > stem bark (70% acetone , wet season) > Leaf (water,wet season) >stem bark (absolute acetone , wet season) > stem bark (water , wet season) > Leaf(water, dry season) >Leaf(absolute acetone, dry season) > leaf (70% acetone , dry season) > Leaf (70% acetone , wet season) > Leaf(absolute acetone , wet season).

The leaf extract of the plant in 70% acetone displayed the maximum in vitro antioxidant activity during the wet season when compared to other parts of the plant during the dry and wet season in all the selected solvents.The absolute acetone stem bark extract of J.Gossypifolia possessed the least in vitro antioxidant activity during the wet season.

Table 4b:The overall trend of total flavonoid and phenolics contents of Jatropha curcas in all the selected solvents

PARAMETER	THE OVERALL TREND OF SELECTED PHYTO CONSTITUENTS ANALYSES
	Decreasing order of bioactivity during wet and dry season using absolute acetone as extraction solvent
Total flavonoid(mg/ml)	Leaf (absolute acetone, dry season) > Leaf (70% acetone , dry season) > Leaf((absolute acetone, wet season) > Leaf (wet season, 70% acetone) >stem bark (absolute acetone ,dry season)> stem bark (70% acetone , dry season) > stem bark (water, dry season) > Leaf (water, wet season > leaf (water, dry season) > stem bark (absolute acetone , wet season) >stem bark (70% acetone,wet season) > stem bark (water, wet season).
Total phenolics(mg/ml)	Leaf(70% acetone, dry season) > Stem bark(70% acetone, dry season) >Leaf(absolute acetone , dry season) > stem bark (Absolute acetone , dry season) > Leaf(water, dry season) > Leaf(70% acetone , wet season) = Leaf(Absolute acetone , wet season) > stem bark(water, dry season) >leaf(water, wet season) > stem(70% acetone, wet season) > stem bark (absolute acetone , wet season) > Stem bark (water, wet season).

The maximum total flavonoid and phenolic contents were observed during the dry season with absolute acetone and 70% acetone as extraction solvents , respectively in all the selected solvents. The total phenolics content of the 70% acetone extract of the plant was equal to the phenolic content of the absolute acetone extract of the same plant during wet season(19.92 ± 0.11 mg/ml)**(Table 4b and 5b).**

Table 5a: Changes in the levels of nitric oxide and antioxidant activities of stem bark and leaf extracts of Jatropha gossypfolia during dry and wet season

SOLVENT	DRY SEASON				WET SEASON			
	Stem bark		Leaf		Stem bark		Leaf	
	Nitric oxide scavenging activity (%)	Antioxidant activity (%)	Nitric oxide scavenging activity (%)	Antioxidant activity (%)	Nitric oxide scavenging activity (%)	Antioxidant activity (%)	Nitric oxide scavenging activity (%)	Antioxidant activity (%)
Water	76.59 ± 0.25	106.12 ± 1.96	13.02 ± 0.43	112.45 ± 1.68	14.08 ± 0.59	71.41 ± 1.82	43.26 ± 1.70	93.80 ± 1.78
70% acetone	62.96 ± 0.26	116.94 ± 0.56	6.98 ± 0.50	113.88 ± 0.56	55.51 ± 0.56	83.60 ± 0.55	2.24 ± 0.46	123.40 ± 1.52
Absolute acetone	59.20 ± 0.26	120.20 ± 1.52	4.72 ± 0.42	115.11 ± 0.85	32.24 ± 2.46	95.40 ± 0.89	1.13 ± 0.19	121.60 ± 0.55

Values are mean ± SD of 5 analyses per sample

The solvent type and concentration modulated the in vitro nitric oxide and antioxidant activities of the stem bark and leaves of Jatropha gossypifolia during the dry and wet season.The highest in vitro antioxidant activity of J . gossypifolia was observed in acetone stem bark extract(120.20%) during the dry season and decreased significantly($P<0.05$) in the same plant part in the same solvent during wet season(95.40%) .In summary, absolute acetone upregulated the in vitro activity antioxidant during the dry season, but down regulated the same parameter during the wet season.Also, 70% acetone stem bark extract of the plant upregulated the antioxidant activity during the dry season(116.94%) , but down regulated the antioxidant activity during the wet season in the same part of the plant(83.60%).

The difference in antioxidant activity of the 70% acetone stem bark extracts during the dry and wet season was significant($P <0.05$).Also, water extract of the stem bark of the plant during the dry season(**106.12%**) was significantly higher($P<0.05$) than wet season (71.41%).The leaf extracts of the plant in dry season were poor scavengers of nitric oxide in vitro,The values of in vitro nitric oxide radical scavenging activity were **3.02 ,** 6.98 and 4.72 % respectively in water , 70% acetone and absolute acetone. The leaf extracts of the plant exhibited potent antioxidant activity in dry season and wet season.

The in vitro antioxidant activity of the leaf extracts of the plant in 70% acetone and absolute acetone were slightly higher in wet season than dry season , but the difference was not significant($P>0.05$).The antioxidant activity of the water extract of the plant in dry season(112.45 %) was significantly higher ($P<0.05$) than the antioxidant activity in wet season(93.80 %).

Table 5b: Changes in the levels of total flavonoid and phenolics of stem bark and leaf extracts of Jatropha gossypifolia during dry and wet season

SOLVENT	DRY SEASON				WET SEASON			
	Stem bark		Leaf		Stem bark		Leaf	
	Total flavonoid (mg/ml)	Total phenolics (mg/ml)	Total flavonoid (mg/ml)	Total phenolics (mg/ml)	Total flavonoid (mg/ml)	Total phenolics (mg/ml)	Total flavonoid (mg/ml)	Total phenolics (mg/ml)
Water	4.52 ± 0.11	14.20 ± 0.11	1.76 ± 0.09	24.40 ± 0.1	0.48 ± 0.11	1.70 ± 0.05	2.52 ± 0.30	5.76 ± 0.09
70% acetone	4.76 ± 0.09	38.36 ± 0.09	16.24 ± 0.33	39.60 ± 0.53	0.72 ± 0.11	5.52 ± 0.11	5.38 ± 0.17	19.92 ± 0.11
Absolute acetone	5.04 ± 0.89	32.32 ± 0.11	17.04 ± 0.09	37.60 ± 0.09	0.96 ± 0.17	5.32 ± 0.11	5.80 ± 0.32	19.92 ± 0.11

Values are mean ± SD of 5 analyses per sample

During the wet season season , the total phenolic content of the leaf of the plant was equal in 70% acetone and absolute acetone extracts(Table 5b).The total phenolic contents of the leaf extracts in dry season in all the solvents(water, 70% acetone and absolute) were significantly higher in dry season than wet season($P<0.05$).The values were (**24.40 , 39.60, 37.60 mg/ml)(wet season) and (5.76, 19.92 19.92 mg/ml)(dry season)** in water, 70% acetone and absolutev acetone , respectively.

Discussion

The nitric oxide scavenging activity of the aqueous extract of *Jatropha gossypifolia* stem bark in the dry season was significantly higher than the antioxidant activity of the aqueous leaf extract of the plant in the wet season($P<0.001$).Also, the in vitro nitric oxide scavenging potential of the stem bark aqueous extract in the dry season was significantly higher than the aqueous leaf extract in the dry season($P<0.001$).The maximum in vitro nitric oxide scavenging activity was 76.59% in the aqueous stem bark extract during the dry season, while the value of the same parameter for aqueous leaf extract was 13.02% activity(Table 1).The maximum in vitro nitric oxide radical scavenging activity for the aqueous stem bark(dry season) and aqueous leaf(wet season) were 76.59 and 14.08 %, respectively.

The ability of an extract to inhibit the formation of nitrite by competing with nitric oxide for oxygen is used to assess its in vitro nitric oxide radical scavenging activity(Marcocci *et al.*, 1994).Scavengers of nitric oxide compete with oxygen to reduce the production of NO (Marcocci et al 1994). The significance of in vitro nitric oxide scavenging activity assay of plant extracts has been hypothesized and experimentally documented (Nabavi *et al.*, 2009).It goes thus: "In vitro nitric oxide scavenging activity assay of plant extracts can candidate them for in vivo nitric oxide scavenging activity''. In our present research, we demonstrated , for the first time, to the best of our knowledge, that the stem bark of *Jatropha gossypifolia* displayed excellent in vitro nitric scavenging activity during the dry season with water as extraction solvent. The in vitro nitric oxide scavenging activity of the 5% aqueous stem bark extract of J.gossypfolia (76.59%) showed less activity when compared to the methanolic extract leaf extract of Alpina malaccensis (80.54%)(Sahoo *et al.*,2012).

This finding observation could be explored by scientists working in the area of immunology for the treatment of immunological disorders. Overproduction of nitric oxide is known as nitrosative stress (Ridnour *et al* ., 2004). Phenolic compounds are important constituents in many plants (Milic *et al.*, 1998).Phenolic compounds behave as antioxidants as a result of the reactivity of the phenolic moiety(Wanasundara and Shaidi, 1998).Plant phenols and flavonoids which are phytochemicals possess radical scavenging activity(Formica and Regalson , 1995).Flavonoids have gained celebrity because of their well demonstrated in vitro antioxidant activity (Rice-Evans *et al.*,1996).DPPH reactivity has been widely used to test the ability of plant extracts to act as free radical scavengers(Liu *et al.*,2008).

In this research work , we have demonstrated unambiguously that 5% absolute acetone stem extract of the plant in dry season exhibited greater in vitro antioxidant potential than ascorbic acid at 1000µg/ml(80.3% activity)(Muthswamy *et al.,* 2012).

The purple colour of DPPH in methanol was bleached to a yellow colour in the presence of plant extracts containing antioxidants(Kumarasamy *et al.,* 2007). DPPH radical scavenging is commonly used substrate for fast evaluation of antioxidant activity because of its stability in the radical form and simplicity of the assay (Bozin et al 2008).The degree of discolouration revealed the scavenging potential of the plant extract(Bhuijan *et al.,* 2009).The antioxidant activity of phenolic compounds is as result of the presence of hydroxyl group which donate proton to free radical and scavenge them (Fukumoto and Mazza , 2010).

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