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Original Article

Studies on *in vitro* Radical Scavenging Potentials of Methanol Leaf Extract of *Ficus sur* and its Fractions

Kelechi Nwabueze Obi*, Eugene Nwaogwugwu Onyeike, Francis Chukwuma Anacletus

ABSTRACT

Background: In many rural areas of Nigeria, *Ficus sur* is traditionally used in the management of many diseases some of whose pathogenesis implicate free radicals. **Objectives:** To determine the phenolic contents and the *in vitro* radical scavenging activities of methanol extracts of *Ficus sur* (MEFS) and its hydromethanol (HMFS), ethyl acetate (EAFS) and hexane (HFFS) fractions. **Materials and Methods:** The leaves of *Ficus sur* were air dried, extracted with absolute methanol, and then partitioned sequentially with hexane and ethyl acetate. The phenolic contents of the parent extract and the fractions were determined. The scavenging activities of the extracts towards 1,1diphenyl-2-picryl hydrazyl (DPPH), hydroxyl, propagating lipid peroxyl and nitric oxide radicals were examined quantitatively. **Results:** Total phenol, flavonols and flavonoids were high in MEFS, moderate in EAFS and low in HFFS. The extracts scavenged DPPH radical (IC_{50} = 42.35± 3.55- 169.43 ± 16.43 , hydroxyl radical (IC_{50} = 55.58 ±8.16- 318.33± 14), peroxyl radical (IC_{50} = 60.33± 13.8- 219± 36.44 µg/ml) and nitric oxide radical (IC_{50} = 41.94 ±6.65- 210.5 ± 11.3 µg/ml) in a dose-dependent manner. The flavonoid contents of the extracts inversely correlated with the IC_{50} values of free radical scavenging activities. Overall, the radical scavenging abilities of MEFS and its fraction follow the order: MEFS> HMFS> EAFS> HFFS. **Conclusion:** Hence, it may be concluded that extracts that have higher phenolic contents are superior to those with lower phenolic contents in radical scavenging activities, which cement the fact that, indeed, phenolics are largely responsible for the invitro antioxidant activities of the plant extracts.

Keywords: *Ficus sur,* Antioxidants, DPPH, Phenolics, Radicals, Scavenging.

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INTRODUCTION

Dependence of most living things on oxygen for oxidation of wide range of substances and as a final electron acceptor during oxidative phosphorylation unavoidably lead to generation of Reactive oxygen species (ROS) and Reactive Nitrogen Species (RNS). Some of these species have unpaired electrons in their outer molecular orbitals and hence very reactive. Some of these reactive species include: hydroxyl radical ($OH^{\cdot}$), superoxide anion ($O_2^{\cdot-}$), Hydrogen peroxide, Nitric oxide (NO) as well as peroxynitirate (ONO_2). The reactive species attack cellular macromolecules (proteins, DNA and lipids) leading to both structural and functional alteration of the later.[1] Consequently, ROS and RNS are implicated primarily or secondarily in pathogenesis of diseases, some of which include: cancer,[2] atherosclerosis,[3] diabetes mellitus,[4] aging,[5] rheumatoid arthritis,[6] HIV-AIDS.[7] Even though ROS and RNS are critical components of diseases, they are used by the immune system to rid human body of invading bacteria. The toxicity of ROS and RNS necessitated the evolution of antioxidant molecules which protect important biological molecules from structural and functional alteration. These protective systems include enzymes such as catalase, superoxide dismutase, glutathione peroxidase or other low molecular weight substances such as ascorbic acid, glutathione, tocopherols and urate.[2] These antioxidants work in synergy to remove ROS and RNS from the tissues.

Aside the endogenously produced antioxidants, Phytochemicals from dietary or herbaceous plants have been shown to have antioxidant properties, and hence modify the pathogenesis of those clinical conditions implicating ROS and RNS.[8-9] The antioxidant activities of plants are mostly attributed to phenolic compounds which have free radical scavenging and metal chelating properties. However, phenolics can be prooxidants at high concentration.[10] The radical scavenging activities of plant phenolics have instigated the search for natural antioxidants as an alternative to synthetic antioxidants such as butylated hydroxyl toluene (BHT) which are under regulatory scrutiny. The aim of this study, therefore, was to determine the phenolic contents and *in vitro* radical scavenging activities of methanol extracts of *Ficus sur* and its hydromethanol, ethyl acetate and hexane fractions.

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Ficus sur (Moraceae) is commonly known as Figure tree. It is a terrestrial and spreading deciduous plant with thick, broad and ever-green leaves.[11] *Ficus sur* produces fruit throughout the year. It is traditionally used in the management of wide range of diseases which include eye problems, diarrhea, wounds, anaemia, among several others. Some reported pharmacological properties of *Ficus sur* include anticancer,[12] immunomodulatory,[13] and antileprosy.[14] These pharmacological properties may be ascribed to wide range of secondary metabolites such as terpenoids, alkaloids, flavonoids, cardiac glycosides, sterols produced by the plant.[15]

MATERIALS AND METHODS

Chemicals and Reagents

Phosphate Buffered Saline, EDTA, hydrogen peroxide, thiobabituric acid, ethanol, trichloroacetate, 1, 1 dipheny 2 pycryl – hydrazyl, methanol, 2-deoxy ribose, iron(III) chloride, L-ascorbate, riboflavin, Nitroblue tetrazolium, hypochloric acid, sodium nitroprusside, sulfanilamide, phosphoric acid, naphtylethylenediamine dihydrochloride, Folin Ciocalteu reagent, Quecertin, Aluminum trichloride, perchloric acid, Gallic acid, hydrogen peroxide, ascorbic acid, butylated hydroxyl toluene. (Were all obtained from sigma Aldrich). sodium carbonate, sodium hydroxide, sodium acetate, sodium nitrite, iron sulphate, potassium dihydrogen phosphate, hydrochloric acid, distilled water, ethylenediamine tetra acetic acid (EDTA), sulphuric acid (were all obtained from BDH).

Plant Materials

The leaves of *Ficus sur* were collected from Abia state Nigeria, identified at the Herbarium of University of Port Harcourt and was issued the voucher number: UPH/P/199.

METHODS

Preparation of Plant Extracts

The plant leaves were air dried to constant weight and then reduced to fine powder by milling. About 200g of each pulverized material were soaked with 1L of absolute methanol and after 48 hr, the extracts were filtered with what man No. 1 filter paper. The filtrate was concentrated with rotary evaporator at reduced pressure, and stored as solids at 4°C for subsequent studies.

Solvent Partitioning

Extraction and partitioning of the extracts were modified from Abu *et al.*[16] Five gram (5g) of MEFS was dissolved with 100ml of 50% methanol and delivered into a separatory funnel. The solution was partitioned sequentially with hexane and ethyl acetate as follows:

Partition: water and hexane. 50ml of hexane was poured into a separation funnel containing 100ml of 50% methanol used to dissolve the solid crude extracts. The separatory funnel was shaken intermittently for 2 min, and allowed to stand for 2hr at room temperature. The aqueous lower layer was run off from the stopcock of the funnel, while the hexane upper layer was poured out of the funnel stopper. This procedure was repeated 5 more times to ensure the exhaustive extraction of hexane soluble components. The resulting hexane extract was filtered and allowed to evaporate at room temperature, and was labelled hexane fraction of *Ficus sur* (HFFS).

Partition: Water and ethyl acetate: Ethyl acetate (50ml) was added to the water layer obtained from the partition with hexane in the separatory funnel. The funnel was shaken intermittently for 2 min, and allowed to stand for 2hr at room temperature. The water layer settled at the bottom was run off from the stop cock, while the ethyl acetate upper layer was collected from the funnel's stopper. The procedure was repeated 5 times to ensure that all ethyl acetate soluble compounds were exhaustively extracted. The resulting ethyl acetate fraction was allowed to evaporate at room temperature. The water fraction was labelled hydromethanol fraction of *Ficus sur* (HFFS) and ethyl acetate fraction was labeled ethyl acetate fraction of *Ficus sur* (EAFS).

Quantitative Phytochemical Analyses

Determination of Total Phenolic Contents

Total phenolics contents of the plant extracts were determined by the modified method of Velioglu *et al.*[17] This is based on the principle that phenolate anion generated under basic condition of the reaction mixture reacts with yellowish Follins-Ciocalteau Reagent (FCR) to produce a blue-coloured product whose intensity is directly proportional to the quantity of phenolics present in the plant extract samples.

One milliliter (1ml) of 1mg/ml plant extracts dissolved in appropriate solvent were added to test tubes, followed by the addition of 5ml of 10% FCR. The mixture was allowed to stand for 5 min at room temperature. Thereafter, 4ml of Na_2CO_3 (60 g/l) solution was added to all the test tubes, and the mixture was left for 90mins. Thereafter, Absorbance at 765 nm was recorded. To determine quantitatively the phenolic contents of the samples, gallic acid was prepared at concentration range of 0-0.3mg/ml and then taken through the same procedure as outlined above for the plant extracts. Standard curve was prepared by plotting absorbance vs concentration, then the quantity of total phenolics (in gallic acid equivalents) in the plant extracts was determine using the following relation

$$C = c.v/m$$

C is total phenolic content (mg/g) in gallic acid equivalent (GAE), c= the concentration (mg/ml) of gallic acid read out from the calibration curve; m= the weight of pure plant extracts and V is the volume of the plant extract solution.

Determination of Flavonoids and Flavonols

The flavonoids content was determined by the modified method of Kumaran and Karunakaran.[18] Flavonoids form a complex with aluminum to generates a yellowish solution whose colour intensity is linearly related to the flavonoid quantity of the samples.

Procedure: one hundred microlitres (100ul) of the 10 mg/ml plant extracts were added to clean test tubes using a micropipette. This was followed by the addition of 100 µl of 20% aluminum trichloride (prepared in methanol) and a drop of concentrated acetic acid. The mixture was then diluted to the final volume of 5 ml with absolute methanol. The mixture was kept at room temperature for 40min, after which absorbance readings was recorded at 415nm using a spectrophotometer. Quercetin at the concentration of 0.5mg/ml was passed through the same treatment as the plant samples and absorbance determined accordingly. The amount of flavonoids in plant extracts (in quercetin equivalents, QE) was calculated using the following formula:

$$\text{Flavonoid content} = \frac{A \times M_0}{A_0 \times M}$$

Where A is the absorbance of plant extract solution, Ao is the absorbance of standard quercetin, Mo is the weight of the plant extracts while M is the weight of the standard quercetin in mg.

The flavonol contents of the extracts were also determined as described by Yermakov *et al.*[19] with slight modifications. Two millilitres (2ml) of 10 mg/ml plant extracts were put in a test tube. This was followed

by the sequential addition of 1ml of 20ml/ml Aluminum Chloride and 3ml of 50mg/ml sodium acetate. The test tubes were left on the laboratory bench for 2 hr. Thereafter, absorbance was determined using a spectrophotometer. To quantify the flavonols in the samples, calibration curve was established by preparing graded (5-100µg/ml) concentrations of the quercetin and taken through the same procedure as the plants extracts. Absorbance at 440nm was finally determined using a spectrophotometer, and the amount of flavonol (in quercetin equivalents, QE) in the plant extract samples was calculated using the formula:

$$X = C.V/m$$

X = flavonol content, mg/g QE, C = concentration of quercetin solution determined from the calibration curve, mg/ml, v= volume of the plant extracts and m =weight of the plant extracts.

In vitro free Radical Assay

Quantitative DPPH radical-scavenging assay

Scavenging activity on DPPH free radicals by the extract was determined using the method of Gyamfi *et al.*[20] with slight modifications.

Briefly, 1.0 ml-solution of two-fold serially diluted extracts (2-500 µg/ml) was added to a series of clean test tubes followed by the addition of 1 ml of freshly prepared 0.3 mM DPPH. The mixtures were shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Blank solutions were prepared with 1 ml each test sample solution and 1ml of methanol, while the negative control sample consisted of 1.0ml of 0.3 mM DPPH solutions and 1 ml of methanol. Thereafter, the absorbance of the assay mixtures was measured at 518 nm using a UV-visible spectrophotometer. The results were compared to ascorbic acid solutions taken through the same procedure as the plant extracts. DPPH radical scavenging activity was determined by the equation:

$$\% \text{ Inhibition} = \left(\frac{A_0 - A}{A_0}\right) \times 100$$

Where Ao is the absorbance of the control and A, the absorbance of the sample. The IC_{50} value represented the concentration of the extract that caused 50% inhibition of DPPH radical and was calculated. All determinations were done in triplicate.

Hydroxyl-radical (·0H)-scavenging Assay

The hydroxyl radical scavenging assay was done using the method described by Halliwell *et al.*[21] with minor modifications. Each reaction mixture contained the following final concentrations of reagents in a final volume of 1.0 ml: 2-dexyribose (2.5 µM)), potassium phosphate buffer (pH 74, 20 mM), $FeCl_3$, (100 µM), EDTA (104 µM), H_20_2 (I mM)) and L-ascorbic acid (100 µM). The mixtures were incubated for 1hr at 37°C. after incubation, 1.0 ml of 1 % (w/v) thiobarbituric acid (TBA) in 0.05 M Na0H and 1.0 ml of 2.8% (w/v) trichloroacetic acid (TCA) were added, and then the resulting mixtures were heated for 15 min at 100°C. After cooling on ice, absorbance was measured at 532 nm. The percentage inhibition of 2-deoxyribose degradation was calculated using the following equation:

$$\% \text{ Inhibition} = \left(\frac{A_0 - A}{A_0}\right) \times 100$$

Where A_0 is the absorbance of the control, and A0 is the absorbance of the tested sample. The IC_{50} value represented the concentration of the extract that caused 50% inhibition. All determinations were done in triplicate.

Lipid Peroxidation Assay

A modified thiobarbituric acid-reactive species (TBARS) assay[22] was used to measure lipid peroxidation in a lipid-rich egg yolk homogenates.[23] This method is based on the principle that malondialdehyde (MDA) generated from peroxidation of polyunsaturated fatty acids in the egg yolk reacts with two molecules of TBA to produce a pinkish chromogen with an absorbance maximum at 532 nm.

Procedure: Five hundred microlitres (500 µl) of 10% Egg homogenate (v/v in phosphate buffered saline (pH 7.4) was added to series of clean test tubes. This was followed by addition of 500 µl of the plant samples (15 –500 µg/ml), 50 µl of 0.075 M $FeSO_4$ and 20 µl of 0.1 M L-ascorbic acid. The mixture was incubated for 1hr at 37°C to induce lipid peroxidation. Thereafter, 0.2 ml of 0.1M EDTA and 1.5 ml of TBA reagent (3 g TBA, 120 g TCA and 10.4 ml 70% $HC10_4$ in 800 ml of distilled water) were added sequentially to the samples and the resulting mixtures heated for 15 min at 100°C, cooled and then pelleted for 10 min at 3000 rpm using a centrifuge. Absorbance of supernatant was measured at 532 nm, and percentage inhibition of lipid peroxidation calculated using the equation:

$$\% \text{ Inhibition} = \left(\frac{A_0 - A}{A_0}\right) \times 100$$

Where A_0 is the absorbance of the control, and A_0 is the absorbance of the tested sample.

All determinations were done in triplicate.

In vitro Nitric Oxide Radical (NO·) Scavenging Assay

The scavenging of NO generated from sodium nitroprusside (SNP) was done according to the method of Marcocci.[24] Reaction mixture (5.0 ml) containing 2.5ml of SNP (5 mM) in phosphate buffered saline (pH 7.3), with or without the 2.5ml of plant extract at different concentrations was incubated at 25°C for 180 min in front of a visible polychromatic light source (25 W tungsten lamp). The NO· radical thus generated interacted with oxygen to produce the nitrite ion ($N0_2$). 1.0 ml of incubation mixture was mixed with 1ml of Griess reagent (1 % sulfanilamide in 5 % phosphoric acid and 0.1 % naphthylethylenediamine dihydrochloride), and absorbance of the resulting purple azo dye was measured at 546 nm. The percentage inhibition was calculated as follows

$$\% \text{ Inhibition} = \left(\frac{A_0 - A}{A_0}\right) \times 100$$

All determinations were done in triplicate.

Data Analysis

The results were analysed by IBM SPSS version 23 (SPSS inc. Chicago, USA). All data are expressed as means ± standard deviation of triplicate determinations. One-way analyses of variance (ANOVA) and Duncan's multiple range test were used to determine the differences among the means. p values < 0.05 were regarded as significant. The IC_{50} values were determined by GraphPad® prism 8. Relationship between flavonoid contents and IC_{50} values was assessed through regression and correlation worked out by using the GraphPad® prism 8.

RESULTS

Quantitative Phenolic Constituents of the Plant Extracts

Table 1 shows the quantitative phenolic contents of MEFS and its hydromethanol (HMFS), ethyl acetate (EAFS) and hexane (HFFS)

Table 1: Phenolic content of MEFS and its fractions.

Extracts fractions	Total phenols (mg/g GAE)	Flavonoids (mg/g Quercetin equiv)	Flavonols (mg/g Quercetin equiv.)
MEFS	209.89±5.80^{d}	42.35±1.43^{a}	18.78±1.10^{a}
HMFS	74.94±4.28^{b}	29.81±1.94^{b}	8.69±0.92^{b}
EAFS	32.71±3.79^{c}	20.26±3.17^{c}	10.19±3.40^{b}
HFFS	20.22±4.64^{a}	16.27±4.33^{c}	2.28±0.62^{c}

Values are Means ± Standard deviation of triplicate determinations.

Values in the same column bearing the same superscript letters are not significantly different at 5% level.

Table 2: Percentage Inhibition for DPPH radical scavenging activities of MEFS and its fractions.

Conc (µg/ml)	MEFS	HMFS	EAFS	HFFS	ASCORBIC ACID
500	83.62±3.50^{b}	60.29±3.03^{c}	59.90±5.05^{c}	50.22±3.28^{d}	91.02±0.43^{a}
250	84.85±3.55^{b}	56.84±3.27^{c}	50.28±0.60^{d}	39.30±3.19^{e}	92.81±1.57^{a}
125	78.10±1.92^{b}	50.74±2.28^{c}	38.56±3.93^{d}	21.94±1.33^{e}	93.28±0.62^{a}
62.5	59.62±2.09^{b}	36.82±4.90^{c}	21.66±5.02^{d}	14.32±2.66^{e}	92.91±1.29^{a}
31.25	41.96±8.42^{b}	20.14±1.02^{c}	12.30±1.69^{c}	12.09±3.86^{c}	86.92±5.84^{a}
15.63	29.67±9.60^{b}	16.00±0.32^{c}	8.04±3.04^{c}	---	43.22±4.22^{a}
IC_{50}	42.35±3.55^{d}	64.78±13.17^{c}	102.33±14.01^{b}	169.43±16.43^{a}	18.36±2.19^{e}

Values are Means ± Standard deviation of triplicate determinations.
Values in the same row bearing the same superscript letters are not significantly different at 5% level.

fractions. Total phenolic, flavonoid and flavonol contents of MEFS and its fractions follow the order: MEFS > HMFS> EAFS > HFFS.

DPPH Radical Scavenging Activity

Table 2 below shows the DPPH scavenging activity of the plant extracts compared to ascorbic acid standard. The scavenging of DPPH was dose-dependent. Ascorbic acid had the highest scavenging activity while HFFS had the lowest scavenging activity. Based on the IC_{50} values the relative order of potencies for DPPH radical scavenging is: Ascorbate> MEFS > HMFS > EAFS >HFFS.

Hydroxyl Radical Scavenging Activities of the Extracts

Table 3 shows the dose dependent hydroxyl radical scavenging activities of the plant extract compared to BHT standard. At concentration of 250µg/ml, MEFS was the most effective (76.92%) while HFFS was the least effective (50.22%). The relative order potency for hydroxyl radical scavenging is: MEFS > HMFS > BHT> EAFS > HFFS.

Lipid Peroxidation Inhibitory Activities of MEFS and its Fractions

Table 4 shows the moderate lipid peroxidation inhibitory activities of MEFS and its fractions compared to BHT reference compound. The inhibition of lipid peroxidation was dose- dependent. At 500µg/ml, MEFS was the most effective (51.17%) while HFFS had the least scavenging activity (28.46). Based on the IC_{50} values, the relative order of potencies for inhibition of lipid peroxidation is: MEFS > BHT> HMFS > EAFS >HFFS.

Table 3: % inhibition values for hydroxyl radical scavenging activity of MEFS and its fractions.

Conc (µg/ml)	MEFS	HMFS	EAFS	HFFS	BHT
250	76.72±3.95^{a}	48.06±0.33^{b}	36.69±0.73^{c}	24.13±0.81^{d}	46.99±3.95^{b}
100	62.80±8.63^{a}	33.75±6.20^{b}	21.45±1.87^{c}	8.08±0.81^{d}	36.08±2.42^{b}
50	36.90±8.64^{a}	17.33±0.76^{c}	10.40±2.36^{cd}	4.46±1.90^{e}	25.90±1.01^{b}
25	30.01±4.88^{a}	4.50±2.31^{c}	6.62±0.32^{c}	3.77±1.65^{c}	17.82±0.74^{b}
10	13.54±3.43^{b}	4.90±1.78^{c}	5.10±1.50^{c}	----	18.12±0.71^{a}
IC_{50}	55.58±8.16^{c}	76.73±9.09^{bc}	102.54±23.92^{b}	318.33±13.50^{a}	97.47±10.98^{b}

Values are Means ± Standard deviation of triplicate determinations.

Values in the same row bearing the same superscript letters are not significantly different at 5% level.

Table 4: Percentage Inhibition for lipid peroxidation inhibitory activities of MEFS and its fractions.

Conc (µg/ml)	MEFS	HMFS	EAFS	HFFS	BHT
250	48.23±0.80^{a}	39.53±1.53^{b}	34.36±0.97^{c}	22.86±2.69^{d}	45.80±0.87^{a}
125	44.85±1.59^{a}	26.58±0.14^{c}	19.53±3.16^{cd}	14.38±1.86^{d}	34.89±5.63^{b}
62.5	36.67±0.51^{a}	21.89±2.03^{b}	10.82±3.64^{c}	8.78±1.38^{c}	25.88±4.40^{b}
31.25	28.18±2.96^{a}	11.71±3.84^{bc}	5.21±2.19^{c}	9.58±3.00^{bc}	14.97±3.34^{b}
15.63	24.07±5.1^{3}a	10.87±1.54^{b}	------	----	12.52±1.61^{a}
IC_{50}	60.33±13.77^{c}	107.2±5.37^{bc}	143.6±6.08^{b}	219±51.48^{a}	85.48±15.03^{bc}

Values are Means ± Standard deviation of triplicate determinations.

Values in the same row bearing the same superscript letters are not significantly different at 5% level.

Nitric Oxide Radical Scavenging Activities of the Extracts

Table 5 below shows the nitric oxide (NO) scavenging activity of MEFS and its fraction compared to ascorbic acid standard. The scavenging of NO was dose- dependent. At extract concentration of 500 µg/ml, ascorbic acid had the highest scavenging activity (91.91%) while HFFS had the lowest scavenging activity (33.02%). Based on the IC_{50} values the relative order of potencies for NO radical scavenging is: Ascorbic acid > MEFS > HMFS > EAFS >HFFS.

The Correlation of Phytochemical Content with IC_{50} Values of Radical Scavenging

Figures 1-4 depict the relationship between the IC_{50} values for radical scavenging activities and phenolic contents of MEFS and its fractions. Results show an inverse correlation between flavonoid contents of the extracts and the IC_{50} values for DPPH, superoxide anion, hydroxyl scavenging abilities of the extracts.

DISCUSSION

Methanol extract of *Ficus sur* (MEFS) and its fractions used in this study contain phenolic compounds. Quantitative phytochemical analyses show that total phenolic content was high in MEFS contents, moderate for HFFs

Table 5: % inhibition values for nitric oxide radical scavenging activity of MEFS and its fraction.

Conc (µg/ml)	MEFS	HMFS	EAFS	HFFS	ASCORBATE
500	64.04±2.29^{b}	57.96±3.85^{b}	42.09±1.70^{c}	33.02±2.89^{d}	91.91±1.25^{a}
250	59.88±2.82^{b}	46.72±5.81^{c}	34.21±6.69^{d}	22.46±3.35^{e}	93.20±1.44^{a}
125	56.01±2.57^{b}	41.26±0.69^{c}	22.90±1.04^{d}	6.83±1.73^{e}	84.62±1.58^{a}
62.5	45.84±1.98^{b}	33.55±2.40^{c}	19.03±0.19^{d}	4.84±2.71^{e}	79.93±0.43^{a}
31.25	32.57±5.37^{b}	21.89±4.89^{c}	8.57±3.08^{d}	2.11±1.40^{d}	64.47±2.22^{a}
15.63	18.02±0.85^{b}	11.85±1.84^{bc}	6.36±0.48^{c}	--	50.07±4.00^{a}
IC_{50}	41.94±6.65^{cd}	73.75±12.98^{c}	161.25±29.63^{b}	210.5±11.31^{a}	28.63±2.95^{d}

Values are Means ± Standard deviation of triplicate determinations.

Values in the same row bearing the same superscript letters are not significantly different at 5% level.

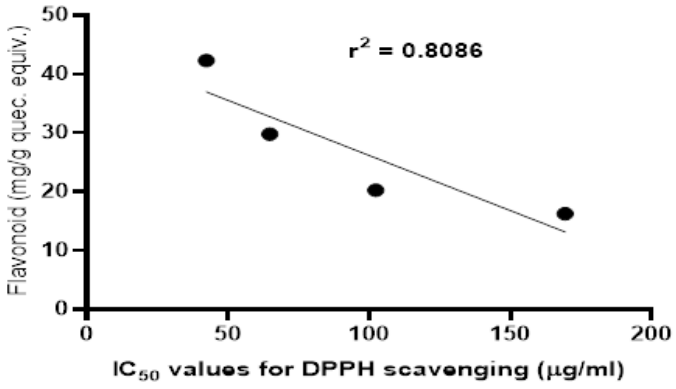

Figure 1: Relationship between flavonoids contents and IC_{50} values of DPPH scavenging activities of MEFS and its fractions.

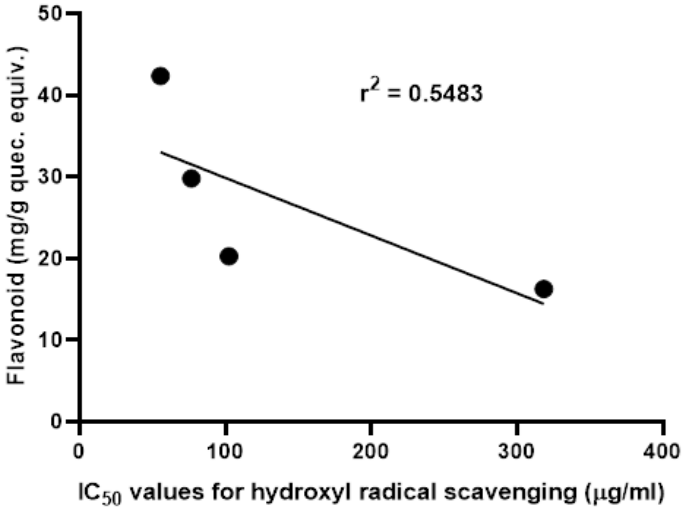

Figure 2: Relationship between flavonoids contents and IC_{50} values of hydroxyl radical scavenging activities of MEFS and its fractions.

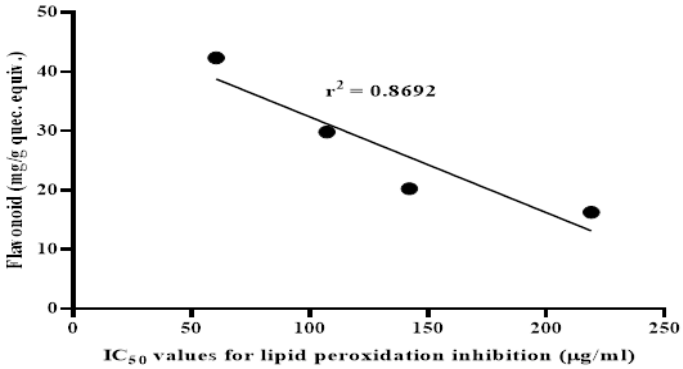

Figure 3: Relationship between flavonoids contents and IC_{50} values for lipid peroxidation inhition activities of MEFS and its fractions.

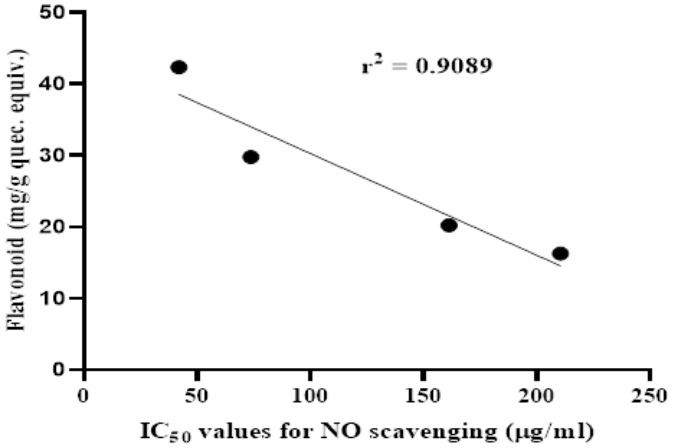

Figure 4: Relationship between flavonoids contents and IC_{50} values of Nitric Oxide scavenging activities of MEFS and its fractions.

and EAFS, and low HFFS. Flavonoid content also followed similar order (Table 1). The flavonol content of MEFS was significantly higher than those of the HMFS, EAFS and MFFS fractions which did no vary widely from one another. The decrease in the phenolic contents of ethyl acetate and hexane fractions may be due to moderate and low polarity of the solvents respectively. Phenolics contains multiple hydroxyl groups which increase their polarity; hence, would not partition into hydrophobic solvents such as hexane to a large extent. Phenolic compounds are found in virtually all plants and plant parts.[25] The ability of phenolics to delay, retard or prevent free radical mediated oxidation of substrates is well known.[26] The free radical scavenging activities of plant extracts are due to their ability to donate hydrogen or scavenge singlet oxygen[27-28] leading to formation of more stable phenoxy radical.[29] Polyphenolic compounds are therefore likely to be responsible for the antioxidant properties of the *Ficus sur* and its fractions observed in this study.

DPPH is a stable free radical used for *in vitro* assessment of qualitative[30] and quantitative[31] antioxidant activities of plant extracts. In this study, the ability of the extract to scavenge DPPH radical was investigated at various concentrations of the extracts, and the result compared to ascorbic (Table 2). Results show that DPPH scavenging activities of the extract was dose-dependent and, on the basis of potency, can be arranged in the following order: MEFS > HMFS > EAFS > HFFS, which was consistent with the phenolic contents of the extracts presented in

Table 1. The decolorization of purple DPPH by MEFS and its fractions is a reflection of hydrogen or electron donating abilities of the plant extracts and, hence, their potential antioxidant activities. In support of Awa *et al.*[26] and Milauskas *et al.*[32] DPPH scavenging activities of MEFS and its fractions observed in this study may due to phenolic compounds.

Hydroxyl radical is a very reactive oxygen species capable of damaging cellular molecules in its immediate vicinity of production.[2] In the present study, the extracts dose-dependently prevented the hydroxyl radical-induced degradation of 2-deoxyribose (Table 5). The relative order of potency for hydroxyl radical scavenging- MEFS > HMFS> EAFS > HFFS- was found to be consistent with the phenolic contents of the extracts presented in Table 1. Earlier studies have also demonstrated the hydroxyl radical scavenging activities of phenolic-rich plant extracts.[22,28] Given the deleterious effects of hydroxyl radical on cellular structure and hence functions,[2] prevention of its formation via the control of tissue levels of hydrogen peroxide and by chelation of transitions metals are critical for cell survival. Direct trapping of hydroxyl radical or chelating of transition metals by components of MEFS and its fractions make the extracts applicable as nutraceutical in prevention of or amelioration of disease conditions such as arthrosclerosis, or as industrial antioxidant in prevention of deterioration of industrial products since hydroxyl radical is implicated in such conditions.[2]

Lipids play important structural and functional roles in biology. The dose-dependent inhibition of lipid peroxidation by MEFS and its fractions compared to BHT reference compound is presented in Table 4. The relative order of effectiveness- MEFS > HMFS> EAFS > HFFS- was consistent with the phenolic content of the plant extracts presented in Table 1. The inhibition of lipid peroxidation by the extract may be due to combination of entrapment of peroxyl radical or by iron chelation attributable to phenolic and flavonoid contents of the plants.[33] Awa *et al.*[27] also reported the lipid peroxidation inhibitory properties of phenolic rich plants. Since lipid peroxidation plays an important role in pathogenesis of cardiovascular diseases, cancer, neurological disorders, and rancidity of lipid-containing industrial products.[34] components of MEFS may find use in the control of such undesired conditions.

The production of nitric oxide (NO) at high level leads to tissue injury and vascular complication. NO reacts with superoxide anion to produce peroxinitrate which finally decompose to hydroxyl radical (HO) and NO_2.[35-36] In this study, the dose-dependent scavenging abilities of the extracts for NO are presented in Table 5. The results are compared with ascorbic acid as a reference compound. MEFS had a good nitric oxide scavenging activity. The potencies of the extract samples may be arranged in the following order: MEFS > HMFS > EAFS > HFFS. This order was equally consistent with their phenolic contents (Table 3). The conclusion that phenolics may be responsible for the nitric oxide scavenging is buttressed by the facts that other phenolic- rich plant extracts were also shown to be effective in nitric oxide scavenging.[37] Since NO is implicated in the pathogenesis of some diseases,[37] MEFS or its fractions may find use in such conditions.

Correlation studies may help to cement the facts that phenolics are the major contributors of *in vitro* radical scavenging activities of plant extracts. In this study, Figures 1-4 show that the flavonoid (the major antioxidant phenolic in phenolic-rich plants) concentration inversely correlated with IC_{50} for DPPH, Hydroxyl nitric oxide scavenging and lipid peroxidation inhibitory activities of the extracts. The observed inverse correlation was due to the fact that fractions with high phenolic contents have smaller IC_{50} value, and hence more potent radical scavenging ability than extracts with lower phenolic contents and higher IC_{50} values. Previous studies have also established similar correlations for phenolic-rich plant extracts.[16,38] It may, therefore, be concluded that phenolics in MEFS and its fractions are largely responsible for the scavenging of the radical species or their precursors.

CONCLUSION

This study shows that radical species generated in the aqueous phase can be scavenged by MEFS and its fractions. The scavenging activity was due to the bioactive phenolic compounds that are known to be good scavengers of radical species. Since activated oxygen intermediate are implicated in the pathogenesis of several diseases as well as in the deterioration of industrial products, it could be emphasized that MEFS can serve as useful sources of nutraceuticals for amelioration disease in which radical species might play important roles or for replacement of synthetic antioxidant (e.g. BHT or BHA) which are still under regulatory scrutiny.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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