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Original article

In vitro profiling of plants used in Sudanese traditional medicine for antioxidant and anti-breast cancer activities

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Abstract

Traditionally, medicinal plants have inhabited an important position in the socio-cultural, spiritual and medicinal domain of rural and tribal lives in Sudan. In the recent years, there is an ever-increasing curiosity in elucidating the role of free radicals in biology, because of their involvement in a diverse range of diseases. Therefore, medicinal plants with antioxidant activity appear to be an intriguing area for research. Hence, the study was aimed to evaluate the antioxidant activity together with anti-breast cancer activity for 14 selected medicinal plants used in Sudanese folkloric medicine. The phytoconstituents of ethanolic extracts were assessed using standard phytochemical screening method. The extracts were then subjected to assess the quantitative antioxidant activity using DPPH radical scavenging and iron chelating assays. MCF7 cell line was used to evaluate the antibreast cancer activity for the most powerful antioxidant extracts. Plant extracts of different concentrations; 5, 12.5, 25, 50 µg/ml were used for the calculation of IC_{50} values using GRAPH PAD PRISM 5 SOFTWARE. Doxorubicin was used as the standard. All plants under study showed positive results for alkaloids, flavonoids, sterols and carbohydrate. *L. inermis*, *L. camara*, *H. abyssinica*, *G. villosa*, and *R. communis*, showed the highest DPPH radical scavenging activity with % RSA values of 93 ± 0.01, 92 ± 0.01, 74 ± 0.14, 61 ± 0.04, and 45 ± 0.02, respectively. On the other hand, ethanolic extracts of *T. foenum-graecum*, *C. decidua*, *B. oralceae*, *A. bracteolata*, and *R. communis* were found to be the top most extracts in terms of iron chelating ability with percentage iron chelating ability values of 68 ± 0.03, 50 ± 0.01, 46 ± 0.10, 44 ± 0.01 and 38 ± 0.02, respectively. All extracts of the selected plants showed marked cytotoxicity against MCF7 cells compared with reference control drug (Doxorubicin). However, among the top highest extracts showing antioxidant activity, *Lawsonia inermis* and *Aristolochia bracteolata* extracts reported the most potent cytotoxic activity with IC_{50} of 19.1 µg/ml.

Keywords: Phytochemical screening, antioxidant, antibreast cancer, Sudanese medicinal plants

1. Introduction

Oxidative stress represents an imbalance between the production and manifestation of reactive oxygen species (ROS) (Lim and Luderer, 2011). These ROS are capable of attacking and damaging the biological molecules such as DNA, proteins, carbohydrates and lipids (Young and Woodside, 2001).The factors causing oxidative stress may be exogenous such as drugs (Gospodaryov and Lushchak, 2012) or endogenous including different cellular organelles such as mitochondria, peroxisomes and endoplasmic reticulum, where the oxygen consumption is high (Phaniendra *et al*., 2015).

The recent years have witnessed an upsurge in studying the role of free radicals in biology, because of their crucial role in various physiological conditions as well as their involvement in a diverse range of diseases (Phaniendra *et al*., 2015) like ischemia-reperfusion injury (Omar *et al*., 1991), coronary arteriosclerosis (Jackson *et al*., 1993), diabetes mellitus (Sugawara *et al*., 1992), neuro degenerative diseases (Simonian and Coyle, 1996), ageing process (Lim and Luderer, 2011) and carcinogenesis (Smith, 1995). ROS are thought to be implicated in cancer pathogenesis through two possible mechanisms: gene mutations and effects on signal transduction and transcription factors (Klatt, 1999; Reynaert *et al*., 2006). The complex antioxidant defense system of the human body consists of exogenous dietary intake of antioxidants, as well as the endogenous production of antioxidant compounds, such as glutathione (Clarkson and Thompson, 2000), that may work either alone, or in association with each other. For example, the combination of vitamin C and vitamin E suppress the formation of hydroperoxide, while metal complex antioxidant such as penicillamine inhibits free radical formation in lipid peroxidation (Feher *et al*., 1987).

Cancer constitutes a major public health problem in many parts of the world (Hanahan and Weinberg, 2000). It is the second most

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common cause of death in the United States, after cardiovascular diseases (Anonyms, 2015) and the third leading cause of death after malaria and viral pneumonia in Sudanese hospital (Anonyms, 2005). In Sudan, like other parts of the world, incidence of cancer has increased phenomenally over the last five decades (Mohammed *et al*., 2013) and breast cancer is the most common type affecting both sex (Ferlay *et al*., 2015). The cost of cancer treatment is humongous, as compared to the success rate of chemotherapy, which is very low. The herbal treatment for breast and prostate cancer showed an 85% success rate at 4 years as compared to the traditional chemotherapy having 2.1% success rate at 5 years (Morgan *et al*., 2004).

Over its long history, the Sudan has witnessed blend of many cultures, Pharonic, Christian and Islamic along with the local indigenous cultures. With this unique history and vast variety of climate and flora, traditional medicine together with medicinal plants became an important part of the cultural heritage of the Sudan (Musa *et al*., 2011).

Medicinal plants, with antioxidant activity containing flavonoids, phenolic acids (Milivojeviæ *et al*., 2011), tannins, anthocyanins (Dyduch-Siemiñska *et al*., 2015), coumarins, xanthones (Kattappagari *et al*., 2015), terpenoids (Tringali, 2001), phenylpropanoids and phenylpropanoid derivatives (Nagababu and Lakshmaiah, 1992) are the major phytoconstituents (Fabricant and Farnsworth, 2001; Rafieian-Kopae*et al*., 2014; Shirzad *et al*., 2011) presenting potential candidature for drug development. In the present work, fundamental initial screening of fourteen selected Sudanese medicinal plants has been carried out for their antioxidant and anti-breast cancer activities *in vitro*.

2. Materials and Methods

2.1 Plant materials

Different parts of fourteen Sudanese medicinal plants (Table 1) were collected in the period, July-December, 2013 from different parts of Sudan. The collected plant parts were authenticated at the herbarium of the Aromatic and Medicinal Plants Research Institute. Voucher specimens were deposited there for future reference.

Table 1: Sudanese medicinal plants

Botanical name	Local name	Part used
Aristolochia bracteolata	AM-Galagil	Roots
Brassica oleracea	Kroomb	Leaves
Capparis decidua	Tondoob	Arial parts
Chamomilla recutita	Al-babonj	Flowers
Cymbopogon citratus	HashishaAllymon	Arial parts
Grewia villosa Willd	Tickoo	Leaves
Hydnora abyssinica	Tratous	Rhizome
Kigelia Africana	Am-Shotor	Fruits
Lantana camara	Lantana	Roses
Lawsonia inermis	Henna	Leaves
Moringa oleifera	Moringa	Roots
Nigella sativa	Habaalsoda	Seeds
Ricinus communis	Kheriwa	Leaves
Trigonella foenum-graecum	Helba	Seeds

2.2 Extraction procedure

The samples were air-dried for two weeks and powdered by a mechanical grinder. 100 g of each plant powder was extracted excessively with 75 % ethanol using Soxhlet extractor for 3 hours. The plant extracts were filtered through Whatman filter paper number 2, and concentrated under reduced pressure and then kept in refrigerator for further investigation.

2.3 Qualitative analysis of phytoconstituents

A portion of the concentrated extract was used for the screening tests using standard method (Ayeni and Yahaya, 2010).

2.3.1 Test for carbohydrates / reducing sugars

The dried powdered plant (5 mg) was boiled with 100 ml of distilled water and the resultant aqueous solution was filtered through a cloth of muslin and tested as follows:

- 2 ml aliquot of the filtrate was mixed with 0.2 ml of ethanolica-naphthol (20%), followed by 2 ml of sulphuric acid (98%), poured carefully on the side of the test tube to form two layers. A violet zone at the junction of the two layers indicated the presence of carbohydrates (Molish's test).
- 5 ml from the filtrate was heated in water bath with 5 ml Fehling's solution for 5 min, a red precipitate indicated the presence of reducing sugars (Fehling test).

2.3.2 Test for tannins

One gram from the dried plant powder was extracted with aqueous ethanol (50%). 5 ml of the aqueous extracts of each plant were added to 0.5 g of NaH_2PO_4, and warmed and then cooled and filtered. To the filtrate, 2 ml of 2% solution of phenazonewas added, resulting in the formation of a precipitate or turbidity thereby, indicating the presence of tannins.

2.3.3 Tests for alkaloids and / or nitrogenous bases

The powdered plant (10 mg) was extracted with acidified water and the acidic filtrate was rendered alkaline by the addition of ammonium hydroxide solution. The mixture was extracted with chloroform and the extract was evaporated to dryness and the residue was dissolved in 2 ml of dilute HCL and tested with the following reagents: Mayer's, modified Dragendorffs and iodoplatinate modified reagent. A white or orange precipitate or violet colour apperared, respectively, with the reagents, indicating the presence of alkaloids and / or nitrogenous bases.

2.3.4 Test for flavonoids

Around 1.0 ml of 10% ethanolic extract of each plant was treated with 0.5 ml of 10% hydrochloric acid, then magnesium metal strips were added. A red colour developed indicating the presence of flavonoids.

2.3.5 Test for saponins

The dried plant powder (1.0 gm) was boiled in 10 ml water for a few minutes, filtered and shaked. A persistent froth indicated the presence of saponins (Froth test).

2.3.6 Test for unsaturated sterols and/or triterpenes

The dried powdered plant (1.0 g) was extracted with few millilitres of ethanol, filtered and evaporated to dryness then the residue was dissolved in distilled water. Acetic anhydride (1.0 ml) and concentrated sulphuric acid (2.0 ml) were added along the side of the tube. A reddish violet colour at the junction of the two layers

indicated the presence of unsaturated sterols and/or triterpenes (Liebermann-burchard's test).

2.3.7 Test for coumarins

A small amount of moistened plant sample was placed in a test tube and covered with filter paper moistened with dilute sodium hydroxide solution. The tube was then placed in boiling water bath for several minutes. The paper was removed and exposed to ultraviolet light. Appearance of yellow to green colour within a few minutes indicates the presence of coumarins.

2.3.8 Test for cardiac-glycoside

One gram of the powdered sample was boiled with 10 ml of 70% alcohol for 5 min on a water bath and filtered. The cooled filtrate was diluted with equal volume of distilled water and a few drops of lead acetate was added and shaken thoroughly. This was allowed to stand for some minutes and then filtered. The filtrate was extracted with 2 volumes of chloroform and the combined extracts were concentrated to form a residue that was used for following test:

- 0.5 gm of the residue was dissolved in 5.0 ml pyridine, followed by addition of 2 drops of 2% sodium nitro-prusside and 2 drops of 20% sodium hydroxide. A deep red color faded to brown indicates presence of cardenolide (Legal's test).

2.3.9 Test for anthracenoside

Three ml of extract was treated with dilute sulfuric acid then boiled and filtered. Equal volume of chloroform was added to the cold filtrate and shaked for some time. The organic layer is separated and treated with 1-2 ml of dilute ammonia (25%). Appearance of pinkish color in ammonia layer indicated anthraquinone glycoside (Borntrager·s test).

2.4 Quantitative determination of the antioxidant activity

2.4.1 DPPH radical scavenging assay

The test was performed according to the method prescribed by Shimada. *et al*. (1992) with some modifications. In a 96-well plate, 10 µl from a 5 mg/ml of each test extract, dissolved in neat dimethyl sulfoxide (DMSO) was allowed to react with 90 µl (300 µM) ethanolic solution of DPPH for half an hour at 37ºC. After incubation, the absorbance of the reaction mixture was measured at 517 nm against a blank solution of DPPH in DMSO using multi-plate reader spectrophotometer. Propylgalate was used as a standard drug at a concentration of 0.5 mM. The assay was performed in triplicate and percentage radical scavenging activity (% RSA) of each test sample was calculated using the following formula:

$$\% \text{ RSA} = [(A_c - A_t)/ Ac] \times 100$$

where, RSA is radical scavenging activity present; A_c is the absorbance of the control and A_t is absorbance of the test extract at 517 nm.

2.4.2 Iron chelating activity assay

The assay was accomplished according to the modified method of Dinis *et al.* (1994). The antioxidant property of the plant extract is demonstrated by their ability to chelate ferrous and disturbing the complex formation between ferrozine and Fe^{+2} which causes a decrease in the red color intensity of the ferrozine - Fe^{+2} complex. In a 96-well plate, 10 µl from a 5 mg/ml, of each test extract, dissolved in ethanol was mixed and allowed to react with Ferrous Sulphate (30 µl, 0.0625 mM in DMSO) for 5 min at room temperature. The reaction was initiated by adding ferrozine (60 µl, 0.5 mM in DMSO). The reaction mixture was mixed thoroughly and left in dark at room temperature for another 10 min. The absorbance of the solution was measured spectrohotometrically at 562 nm. Ethylene diamine tetra-acetate (EDTA, 0.5 mM) was used as standard and DMSO as control. The assay was performed in triplicates and the percentage inhibition of ferrozine-Fe^{+2} Complex was calculated using the following formula:

Ferrous ion-chelating ability (%) = [(Abs control-Abs sample)/ Abs control] × 100.

2.5 Screening anti-breast cancer activity

Cell line (MCF7) was obtained from the American Type Culture Collection (ATCC, Minnesota, U.S.A.), and the activity was performed as per the method by Skehen *et al.* (1990).

2.5.1 Cell culture

MCF7 cells were grown at 37°C in a humidified atmosphere containing 5% CO_2 (Heraeus BBD 6220 incubator) and cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), and 1% penicillin/streptomycin. For experiments, frozen cells in cryotubes were kept in liquid nitrogen at-180°C and thawed in a water bath at 37°C. After thawing, the contents were supplied to a 5 ml culture medium in a 50 ml sterile falcon tubes. Cells were then incubated at 37°C for 2 h, and centrifuged at 1200 rpm for 10 min. Afterwards, the cell pellet was suspended in warm culture medium and seeded in 5 ml supplemented medium in T25 nunclon sterile tissue culture flask. Thereafter, cell suspension was incubated at 37°C in a humidified atmosphere containing 5% CO_2. Medium was changed every 2-3 days and continued to incubate the cells in same conditions until a confluent growth. Cells were then washed twice in phosphate buffered saline (PBS) and subsequently incubated with trypsin-EDTA solution for 2 min. at 37°C. After cells had dislodged from the surface, the culture medium was added, and centrifuged at 800 × g for 5 min (Heraeus Megafuge 1.0, rotor 7570F). Then, the cell pellets were re-suspended in warm culture medium, diluted and transferred to sterile culture flasks. Cell numbers were determined by using Neubauer chamber. The amount of dead cells were determined by Trypan blue exclusion. For long-term storage, the cells were treated as follows:

After trypsinization, cells were pelleted (5 min at 400 × g), diluted in freezing medium (growth medium with 20% FBS supplemented with 10% DMSO) and stored in sterile cryotubes (Nunc). The cryotubes were gradually cooled down from 0°C to –80°C and finally, stored in liquid nitrogen (–180°C) till use.

2.5.2 Determination anti-breast cancer activity of the plants understudy

The plant extract was prepared in di-methyl sulfoxide (DMSO). Different concentrations of the plants extract were used; 5, 12.5, 25, 50 µg/ml. For determination of cytotoxicity, cells were seeded into a 96-well microtiter plates at concentration of 3×10^3 cell/well in a 150 µl fresh medium and left for 24 h to attach to the plates. Then, different concentrations of plants extract as well as doxorubicin (positive control) were added, each in triplicate. The

plates were incubated at 37 ^{0}C in a humidified atmosphere containing 5% CO_2 for 48 h. Afterwards, the cells were fixed with 50 µl cold trichloroacetic acid (10% final concentration) for 1 h at 4°C. Thereafter, the plates were washed with distilled water and stained with 50 µl 0.4 % sulphorhodamine-B (SRB), dissolved in 1 % acetic acid for 30 min at room temperature. Plates were then washed with 1% acetic acid and air dried. Finally, the dye was solubilized by the addition of 100 µl/well of 10 M tris base (pH 10.5) and the optical density (O.D.) of each well was measured spectrohoto metrically at 570 nm with an ELISA microplate reader (Skehan *et al*., 1990). The mean background absorbance were automatically subtracted and mean values for each drug concentration was calculated. The experiment was repeated 3 times. The percentage of cell survival was measured as follows:

Surviving fraction = O.D. (treated cells)/ O.D. (control cells)

2.6 Statistical analysis

All data are presented as Mean ± standard deviation. The IC_{50} values (the concentrations of the plant extract and the drug (doxorubicin) required to produce 50% inhibition of cell growth) were calculated by using GRAPH PAD PRISM 5 SOFTWARE.

3. Results

3.1 Phytochemicals screening

All plants under study showed positive results for alkaloids, flavonoids, sterols and carbohydrate whereas; saponins were only detected in *Trigonella foenum-graecum, Aristolochia bracteolata* and *Nigella sativa*. On other hand, cardiac glycosides were detected in *Capparis decidua and Kigelia africana*. Coumarin was detected only in *Kigelia africana* while anthracenes were not detected in any of the extracts under study (Table 2).

Table 2: Qualitative analysis of phytoconstituents

Plant	Sap.	Alk.	Flav.	Ster	Carbo.	R.sug.	Tann	Coum	C.Glyco	Anthra
Lawsoniainermis	–	+	+	+	+	–	+	–	–	–
Capparis decidua	–	+	+	+	+	–	+	–	+	–
Hydnoraabyssinica	–	+	+	+	+	+	+	–	–	–
Cymbopogoncitratus	–	+	+	+	+	+	–	–	–	–
Grewiavillosa	–	+	+	+	+	+	–	–	–	–
Kigeliaafricana	–	+	+	+	+	–	–	+	+	–
Brassica oleracea	–	+	+	+	+	–	–	–	–	–
Trigonellafoenum–graecum	+	+	+	+	+	+	+	–	–	–
Lantana camara	–	+	+	+	+	+	+	–	–	–
Aristolochiabracteolata	+	+	+	+	+	–	–	–	–	–
Nigella sativa	+	+	+	+	+	–	+	–	–	–
Ricinuscommunis	–	+	+	+	+	+	–	–	–	–
Moringaoleifera	–	+	+	+	+	–	–	–	–	–
Chamomillarecutita	–	+	+	+	+	–	+	–	–	–

Saponins (Sap.), Alkaloids (Alk.), Flavonoids (Flav.), Sterols (Ster.), Carbohydrate (Carbo.), Reducing sugers (R.sug), Tannins (Tann.), Coumarins (Coum.), Cardiac Glycoside (C.Glyco.), Anthracene (Anthra.),present (+), absent (–).

3.2 Quantitative determination of the antioxidant activity

Ethanolic extracts from *L. inermis, L. camara, H. abyssinica, G. villosa,* and *R. communis,* reported highest DPPH scavenging activity with % RSA values of 93 ± 0.01, 92 ± 0.01, 74 ± 0.14, 61 ± 0.04, and 45 ± 0.02, respectively. Whereas, the highest iron chelating activity was found in the following extracts:

T. foenum-graecum, C. decidua, B. oralceae, A. bracteolata, and *R. communis,* with % iron chelating ability values of 68 ± 0.03, 50 ± 0.01, 46 ± 0.10, 44 ± 0.01 and 38 ± 0.02, respectively (Table 3).

3.3 Anti-breast cancer activity for plants with higher antioxidant activity

The plant extracts showing highest antioxidant activity in both the assays were subjected to *in vitro* testing of cytotoxicity against MCF7 cells. All the extracts tested showed marked cytotoxicity against MCF7 cells as compared with reference to control drug (Doxorubicin). *Lawsonia inermis* and *Aristolochia bracteolata* extracts were reported to possess the most potent cytotoxic activity with IC_{50} of 19.1µg/ml (Table 4) and (Figure1).

4. Discussion

Chemically derived drugs have been developed for cancer treatments. However, the current chemotherapeutic agents have limitations due to their toxic effects on non-targeted cells, resulting in adverse consequences (Seca and Pinto, 2018). Therefore, there is a demand for alternative treatments with plant-derived anticancer agents with less toxicity to normal cells. In addition,in the recent past, interest has increased largely in finding natural antioxidants to replace synthetic antioxidants in foods or medicinal products due to their adverse reaction such as carcinogenicity. The antioxidant activity of herbal plants is said to be due to the presence of phytoconstituents, such as phenols and flavonoids. The polyphenolic compounds are said to have chemopreventive and suppressive activities against cancer cells by inhibiting many metabolic enzymes involved in the activation of potential carcinogens or arresting the cell cycle (Sylvie *et al*., 2014).

Table 3: Free radical (DPPH) scavenging activity and iron chelating ability of the 14 plant extracts

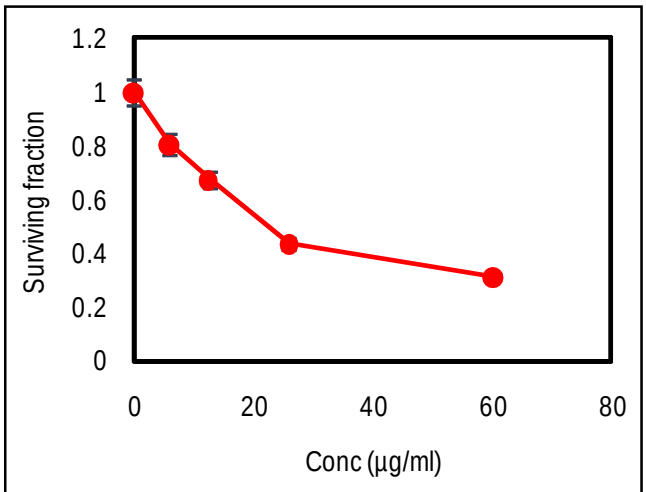
Plant ethanol extracts	% Radical scavenging activity (RSA) ± SD	% Iron chelating ability ± SD
Aristolochia bracteolate	18 ± 0.02	44 ± 0.01
Brassica oleracea	14 ± 0.01	46 ± 0.01
Capparis decidua	08 ± 0.03	50 ± 0.01
Chamomillarecutita	38 ± 0.04	29 ± 0.01
Cymbopogoncitratus	30 ± 0.02	35 ± 0.02
Grewiavillosa	61 ± 0.04	14 ± 0.02
Hydnoraabyssinica	74 ± 0.14	22 ± 0.03
Kigelia Africana	26 ± 0.02	34 ± 0.04
Lantana camara	92 ± 0.01	27 ± 0.01
Lawsoniainermis	93 ± 0.01	32 ± 0.02
Moringaoleifera	35 ± 0.01	24 ± 0.01
Nigella sativa	07 ± 0.03	16 ± 0.01
Ricinuscommunis	45 ± 0.02	38 ± 0.01
Trigonellafoenum-graecum	0.8 ± 0.01	68 ± 0.03

Standard propylgalate = 92 ± 0.01; EDTA = 90 ± .01

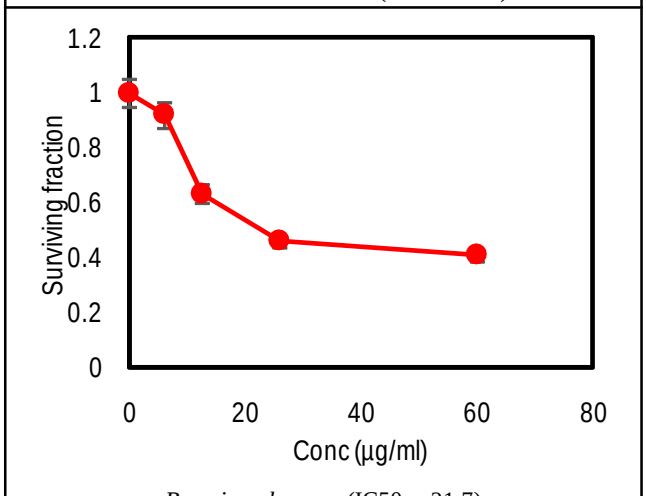
Table 4: IC_{50} in µg/ml for ethanolic extract of selected plants

Plant ethanolic extract	IC_{50} (µg/ml)
Aristolochia bracteolate	19.1
Brassica oleracea	21.7
Capparis decidua	20.3
Cymbopogoncitratus	25.5
Grewiavillosa	21.5
Hydnoraabyssinica	35.5
Lantana camara	20.5
Lawsoniainermis	19.1
Trigonellafoenum-graecum	20.5
Doxorubicin (positive control)	4.2

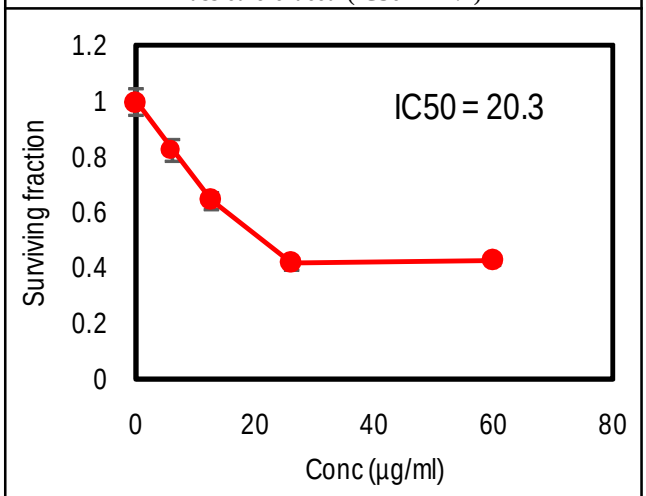
In the present study, fourteen Sudanese plants were extracted and evaluated for their antioxidant and anti-breast cancer activities. The antioxidant activity of the plants extracts was evaluated based on their ability to trap DPPH radical and ability to chelate ferrous. *L. inermis* have shown the strongest reducing antioxidant capacity and free radical scavenging ability using DPPH method and iron chelating assay; and these results are almost in agreement with its flavonoids content. Consequently, the antioxidant activity of this plant might be relatedits phenolic content, as also reported in previous studies (Hsouna *et al.*, 2011; Guha *et al.*, 2011; Kumar *et al.*, 2014). *L. inermis* also exhibited anti-breast cancer activity, suggesting that the free radical scavenging activity might contribute significantly to the anti-breast cancer activity. Several alkaloids isolated from natural herbs exhibit anticancer effects on various types of cancers both *in vitro* and *in vivo* (Iqbal *et al.*, 2017). Alkaloids showed diversity in their mechanism of anticancer effect by modulating multiple signaling pathways, resulting in inhibition of glutathione (GSH), topoisomerase enzymes, extra cellular signal-regulated kinase (ERK) and mitogen-activated protein kinase phosphatase 1 (MKP-1) (Lu *et al.*, 2012). Our study reported presence of alkaloids in *L. inermis*, which may contribute to the anti-breast cancer activity of this plant.



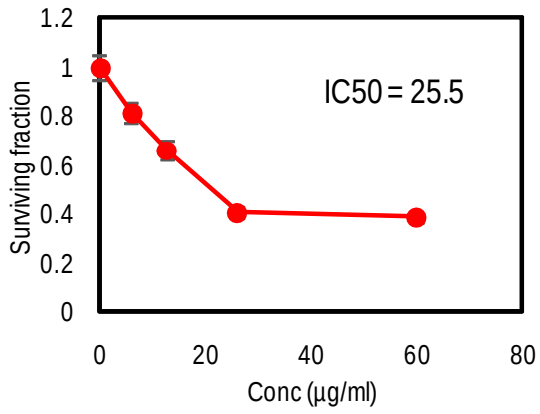
Aristolochia bracteolate (IC50 = 19.1)



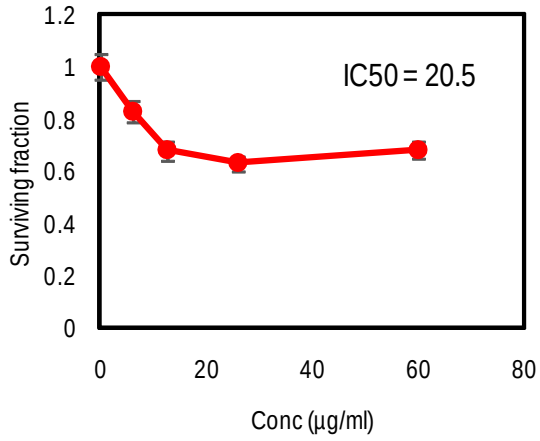
Brassica oleracea (IC50 = 21.7)



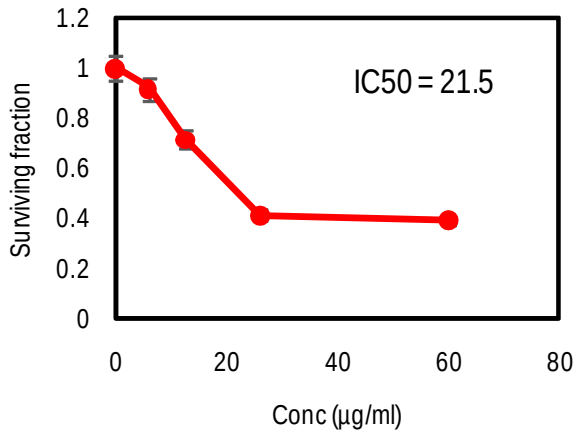
Capparis decidua (IC50 = 20.3)



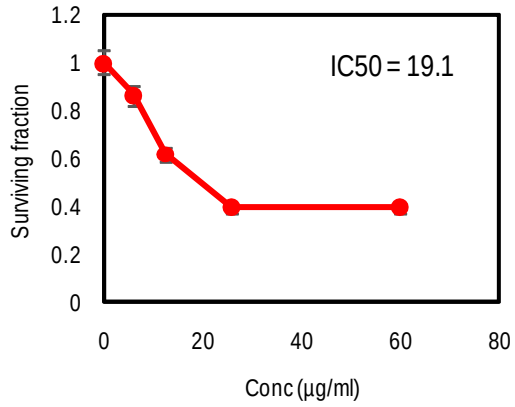
Cymbopogon citratus (IC50 = 25.5)



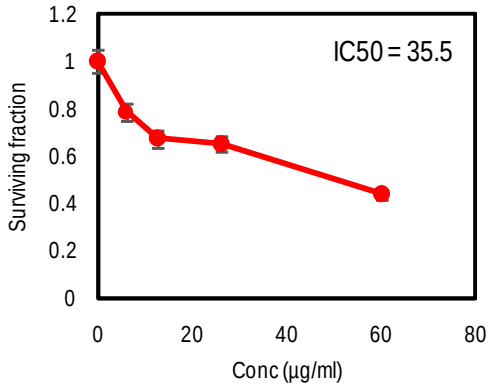
Lantana camara (IC50 = 20.5)



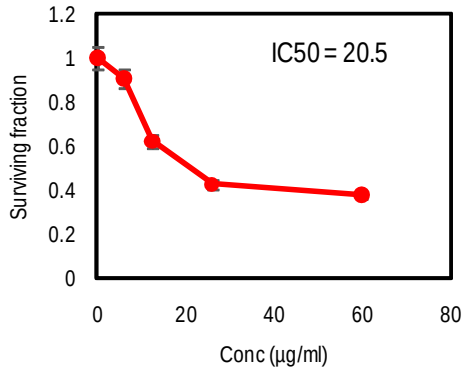
Grewia villosa (IC50 = 21.5)



Lawsonia inermis (IC50 = 19.1)



Hydnora abyssinica (IC50 = 35.5)



Trigonellafoenum-graecum (IC50 = 20.5)

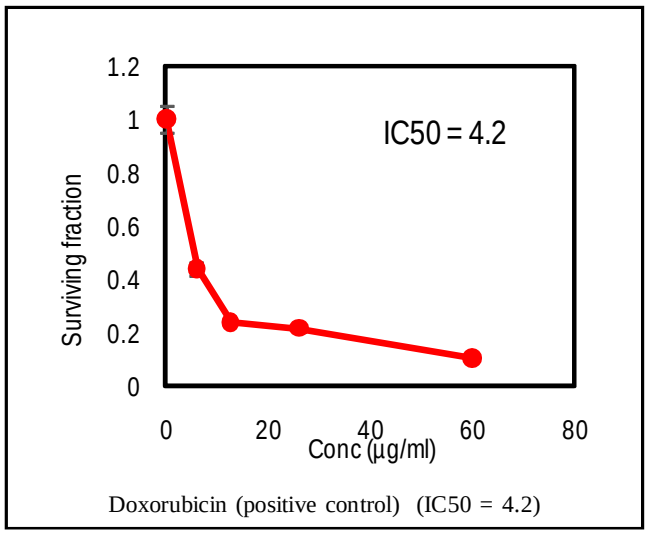


Figure 1: IC_{50} for of selected plants and standard doxorubicin on MCF7 cell line.

Lantana camara showed high antioxidant activity using DPPH method and iron chelating assay. Additionally, this extract exhibited strong anti-breast cancer activity against MCF7 cell line. The presence of polyphenolic compounds, such as flavonoids, contributed to its antioxidant activity while anti-breast cancer activity might be due to the presence of polyphenolics, sterols and alkaloids (Iqbal *et al*., 2017; Lu *et al*., 2012; Bradford and Awad, 2007; Novotny *et al*., 2017).

Aristolochia bracteolate showed pronounced anticancer activity against MCF7 cell line and antioxidant activity using iron-chelating assay. However, the free radical scavenging activity using DPPH radical scavenging assay was only moderate, contrary to a previous study, showing the significant antioxidant activity of ethanolic exract of *Aristolochia bracteolate* using DPPH radical scavenging assay (Eltayeb and Nari, 2017). The potential antioxidant and anticancer activity of this plant might be due to the presence of phytochemicals such as flavonoids, sterols, alkaloids and saponins, which has been reported to play a significant role in inhibiting cancer cell activating proteins, enzymes and signaling pathways (Iqbal *et al*., 2017). Several studies have described the antioxidant and anticancer activity of *Aristolochia* species (Subramaniyan *et al*., 2015; Dirar *et al*., 2014).

Capparis deciduas (arial parts) and *Trigonella foenum-graecum* (seeds) have pronounced antioxidant activity using iron-chelating assay while their activities on scavenging DPPH radicals appeared to be low when compared with other plants. In the current study, *Capparis decidua* and *Trigonella foenum-graecum* exhibited anticancer activity against breast cancer (MCF-7) cells, which could be due to their antioxidant activities. These findings are in agreement with a study in India, where *Capparis spinosa* inhibited proliferation of hepatic (HepG2) cells, colon (HT29) and breast cancer MCF-7 cells (Upadhyay, 2011). The anticancer property of *Trigonella foenum-graecum* seeds might be due to presence of diosgenin, a steroid saponin, which has been reported to inhibit cell growth and induce apoptosis in colon cancer cell line (Raju *et al*., 2004). Additionally, fenugreek exhibited selective toxicity against various cancer cells including breast, pancreatic and prostate cancer cell (Wani and Kumar, 2018).

Other plants such as *Grewia villosa*, *Hydnora abyssinica*, *Cymbopogon citratus* and *Brassica oleracea* showed good antioxidant potential but were not very effective against MCF-7 cells as evident by their higher IC_{50} values.

5. Conclusion

We, thereby conclude that plants such as *Lawsonia innermis*, *Lantana camara*, *Aristolochia bracteolate*, *Trigonella foenum-graecum* and *Capparis decidua* are the promising candidates for use as natural products based antioxidant and anti-breast cancer for human health. As it is a preliminary study, we recommend further studies on these plants to evaluate their potential in *in vivo* and clinical study.

Conflict of interest

The authors declare that no conflict of interest exists in the course of conducting this research. All authors had final decision regarding the manuscript and the decision to submit the findings for publication.

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