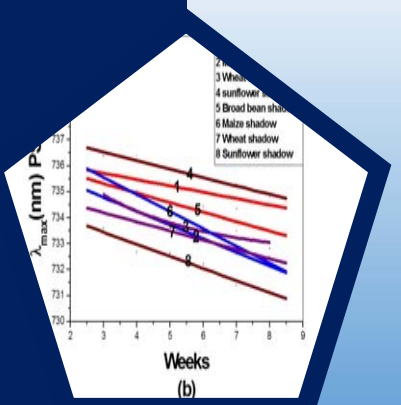
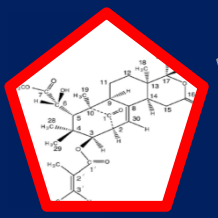
Journal of Faculty of Sciences

A Refereed Scientific Journal
Faculty of Pure and Applied Sciences
International University of Africa
Volume No. 5 , December , 2018





ISSN: 1858 – 513x

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Anticancer and Antioxidant Activity of Five Sudanese Medicinal Plants from the Family Fabaceae

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ABSTRACT

Fabaceae is the third largest family of flowering plants including; herbs, shrubs, trees and vines distributed throughout the world, especially in tropics rain forest and widespread in Sudan. The main objective of study was to screening some Sudanese medicinal plants for anticancer and antioxidant activity to predict their anticancer activity for breast and liver cancers. The leaves of five Sudanese medicinal plants, *Pongamia pinnata*, *Acacia sieberiana, Bauhinia rufescens, Prosopis juliflora*, and *Hardwickia binata* were investigated *in vitro* against two main cancer cell lines; HePG2 (Liver cancer) and MCF_7 (Breast cancer) as well as; against DPPH, FRAP assay and measuring TPC. The petroleum ether and methanol extracts of *Pongamia pinnata* leaves were found the most potent anticancer against both $HePG_2$ and MCF_7 cell lines with IC_{50} 17.4 and 3.8 µg/ml respectively. While leaves petroleum ether extracts revealed lower IC_{50} 41.1 µg/ml against $HePG_2$ cell lines. The methanol extracts of *Hardwickia binata* were found of less anticancer activity against $MCF_7$and $HePG_2$ with IC_{50} 30.8 and 44.9µg/ml respectively. All plant methanol extracts showed high potent ferric reduction antioxidant power (FRAP) 65-99% rather than petroleum ether extracts. The petroleum ether extracts of *A. sieberiana and B. rufescens* leaves potent high anti DPPH with IC_{50} 28.0 µg/ml and IC_{50} 91.6 µg/ml respectively as well as

the methanol extracts of *Hardwickia binata and B. rufescens* with IC_{50} 52.3 µg/ml and IC_{50} 56.8 µg/ml respectively compared to standard Vitamin C (18.5µg/ ml). This result also observed in TPC which ranging from 1.51-7.23 µg/ml in methanol extracts while it ranges from 0.00-7.05 –µg/ml in case of petroleum ether extracts

Keywords: anticancer; antioxidant; Fabaceae family species

مستخلص

عائلة Fabaceae البقوليات تعتبر ثالث أكبر عائلة من النباتات المزهرة بما في ذلك ؛ الأعشاب والشجيرات والأشجار والكروم المنتشرة في جميع أنحاء العالم ، وخاصة في الغابات الاستوائية المطيرة وعلى نطاق واسع في السودان .الهدف الرئيسي من الدراسة هو فحص بعض النباتات الطبية السودانية لمضادات الأكسدة و النشاط المضاد للأكسدة للتنبؤ بنشاطها المضاد للسرطان لسرطان الثدي والكبد . تم فحص أوراق خمسة نباتات طبية سودانية ، البونجاميا *Pongamia pinnata*،*الكاكاموت Acacia sieberiana*، *Bohhinia rufescens خف الجمل ، المسكيت Prosopis juliflora* ، و *Hardwickia binata الهاردويكا في* المختبر ضد اثنين من خطوط الخلايا السرطانية الرئيسية ؛ HePG2(سرطان الكبد) و MCF7 (سرطان الثدي) ، وكذلك ضد DPPH ، اختبار FRAP وقياس .TPC تم العثور على الأثير البترولي ومستخلصات الميثانول من أوراق Pongamia pinnataكأقوى مضاد للسرطان ضد كل من خطوط الخلايا HePG2 و MCF7 مع IC50 17.4 و 3.8 ميكروغرام / مل على التوالي . في حين أن أوراق مستخلصات إيثر النفط قد كشفت عن انخفاض IC50 41.1 ميكروغرام / مل ضد خطوط الخلايا HePG2 و أظهرت مستخلصات الميثانول من Hardwickia binata أقل نشاط مضاد للسرطان ضد MCF7and HePG2 مع IC50 30.8و 44.9 ميكروغرام / مل على التوالي . وأظهرت جميع مستخلصات الميثانول النباتية قدرة قوية مضادة للأكسدة للحد من الحديديك99-65 (FRAP) ٪ بدلا من مستخلصات إيثر البترول. و أظهرت مستخلصات الأثير البترولي لـ A. sieberiana و B. rufescens مقاومة عالية لـ DPPH مع IC50 28.0 µg / ml و IC50 91.6 µg / ml على التوالي بالإضافة إلى مستخلصات الميثانول من Hardwickia binata و B. rufescens مع IC50 52.3 µg / ml و IC50 56.8 µg / mlعلى التوالي مقارنة بفيتامين C القياسي (18.5 ميكروجرام / مل . و هذه النتيجة لوحظت أيضا في TPC والتي تتراوح من 1.51-7.23 ميكروغرام / مل في مستخلصات الميثانول في حين تتراوح من 0.00-7.05 µg / ml-- في حالة مستخلصات إيثر البترول.

1. INTRODUCTION

Cancer diseases become the most health problem in the world and one of the most killer diseases in developing as well as developed countries. The annual global mortality rate due to cancer approximately is, 6 million deaths per year, representing 12% of all deaths worldwide (Srivastava, *et al*., 2005). However global estimates showed that 80% of about 4 billion population cannot afford products of Western Pharmaceutical Industry, and have to rely upon traditional medicines that derived from plant materials. Plants have a long history of use in treatment of cancer and currently the anticancer agents used are derived from natural sources that include; plants, marine organisms and micro-organisms (Rajandeep, *et al.,* 2011).

Traditional systems of medicine continued to be practiced on many accounts due to population rise, inadequate supply of drugs, high cost of treatments and side effects of several allopathic drugs. In addition to development of resistance to currently used drugs for infectious diseases have led to increase emphasis on use of plant materials such leaves as source of medicines for a variety of ailments *(*Joy, 1998*).* Nevertheless many plant-derived products have been shown to exhibit potent anti-tumors activity against several rodent and human cancer cell lines **(**Madhuri & Govind, 2009). Historically pharmacological screening of compounds natural or of synthetic origin has been the source of innumerable therapeutic agents and; random screening as a tool to discover new biological active molecules has been most productive in area of antibiotics potentiality (Mahesh, 2008).

Traditional uses of some plants

Fabaceae is the third largest family of flowering plants with more than 18,000 described species including; herbs, shrubs, trees and vines distributed throughout the world, especially in tropics rain forest and widespread in Sudan (El-Amin, 1990).. The fruits and sprouts of *Pongamia pinnata* were used in folk remedies for tumors. The *P. pinnata* plant is used for anti-inflammatory, antiplasmodial, anti-nonciceptive, anti-hyperglycaemics, anti-lipidoxidative, anti-diarrhoeal, anti-ulcer, antihyperammonic, CNS depressant activity and antioxidant (Ashish *et al.*, 2009). *Acacia sieberiana* DC. The bark, leaves and gums are used to treat tapeworm, bilharzia, haemorrhage, orchitis, colds, diarrhoea, gonorrhoea, kidney problems, syphilis, ophthalmia, rheumatism and disorders of the circulatory system (Wikipedia, 2009). *Bauhinia rufescens* Lam. extracts from the root are used as an astringent or antipyretic in local medicine. Leaves and fruit are applied for the treatment of diarrhea, dysentery and ophthalmic or as tonic .The bark of the root is used to cure chest complaints, syphilis and other venereal diseases, leprosy and reduce fever, the fruit against dysentery (Maydell &Von, 1986).

Prosopis glandulosa has been used for a variety of medicinal purposes including lice control and treatment of sore throat, skin sores and ulcers. Reported to be a collyrium, emetic and laxative, *P.glandulosa* is a folk remedy for dyspepsia, eruptions, hernias and skin and umbilical ailments (AGRO). *Hardwickia binata* The natives of Chhattisgarh Plains used this

leaves for headache. The natives of Kanker region used leaves for purgative and can be used in treatment of constipation. Balsam is used for sexually transmitted diseases and it is similar to Copaiba balsam (*Copaifera langsdorffii* Desf. *Leguminosae*) of Brazil and is used in leucorrhoea, chronic cystitis, gonorrhoea, combinedwith Cubebs and sandal. The resin is used as diuretic and the bark has a good absorption capacity for mercury from water. Seeds are used for dysentery (AGRO). Accordingly the main objective of the study was to screening some of the Sudanese medicinal plants for anticancer, antioxidant activity to predict their anticancer activity for breast and liver cancers.

2. MATERIALS AND METHODS

The selected plant species were collected from their natural habitats in Western and Central Sudan from different localities in 2009. The experiments were conducted at Medicinal and Aromatic Plants Research Institute (MAPRI), Khartoum, Sudan, in the Institute of bioscience, University of Putra Malyasia, Malyasia, Serdang. All the specimens were taxonomically identified by Dr. Wai'l S. Abdalla and Dr. Haidar Abdelgadir members of the Herbarium in Medicinal and Aromatic Plants Research Institute (MAPRI), Khartoum, Sudan, while one plant *Hardwickia binata* was identified in Khartoum University, Department of Botany by Dr. Manal Elhakeem, and the specimens were deposited at the herbarium of MAPRI.

The plant parts were shade dried and the powdered materials were accurately weighed then after were extracted by shaker in room temperature by using the two solvents petroleum ether and methanol respectively. After that the filterated extracts were concentrated by rotatory evaporator to remove the solvent. The extracts, were dried and concentrated by using hood extracts and the yield percentage was then calculated Table (1).

Microculture Tetrazolium (MTT) assay The tumor cell lines were maintained in the Institute of bioscience, University of Putra Malyasia, Malyasia, Serdang**.** The assay is based on capacity of Mitochondria succinate dehydrogenaze enzymes in living cells to reduce yellow water soluble substrate [3-(4, 5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide] into insoluble purple Formazan product which is measured by spectrophotometer and MTT activity level is a measure of viability of cells. The monolayer cell culture was trypsinized and the cell count was adjusted to 1×10 cells/ ml using medium containing 10% fetal bovine serum (FBS). To each of 96 wells micro titer plates, 100µl of diluted cell suspension was added. After 24 hours the supernatant was flicked off and 100µl of fresh medium containing serum was added to all wells, then after seven serial dilution of crude extracts in growth medium was prepared and kept at 37ºC in 5 % CO_2 incubator for 72 hour.

The cells were periodically checked for granularity, shrinkage and swelling. After 72 hour, 50µl of MTT dye was added to each well which

prepared by 5mg/ml concentration. The plates were gently shaken and wrapped in aluminum foil and incubated for 4 hours at 37oC in 5% CO2 incubator. The supernatant was removed, 100µl of DMSO was added, and plates were gently shaken to soluble the formed Formazan. The absorbance was measured using ELISA reader at a wavelength of 570 nm. The percentage growth inhibition was calculated using the formula:

% cell inhibition= 100-{(Ac-At)/Ac} x100; Where, At = Absorbance value of test compound; Ac =Absorbance value of control

The total antioxidant capacity of biological samples was measured by three methods; Ferric-Reducing Antioxidant Power (FRAP) assay that depends on reduction of ferric tripyridyltriazine (Fe (III)-TPTZ) complex to ferrous tripyridyltriazine (Fe (II)-TPTZ) by a reluctant at low pH. (Fe (II)-TPTZ has an intensive blue colour and can be monitored at 593 nm (Benzie and Strain 1996), DPPH and TPC (Total Phenol Compound). FRAP working solution of 25 ml acetate buffer was prepared by 300 mmol/l pH 3.6 (3.1g sodium acetate ×3 H_2O and 16 ml conc. acetic acid per 1 of buffer solution and; 2.5 ml TPTZ solution was prepared by 10 mmol/l 2, 4, 6-tripyridyl-s-triazine (TPTZ) in 40 mmol/ l HCl and; 2.5 ml $FeCl_3$ × 6 H_2O solution prepared by 20 mmol/l FeCl3 x 6 H2O in distilled water.

Preparation of the standard 100µM FeSO4.H2O in (D.W): The fresh working solution was prepared by mixing 25mL acetate buffer, 2.5mL TPTZ

solution, and 2.5mL $FeCl_3$ $6H_2O$ solution and then warmed at 37 C° before using. The extracts (10µL) were allowed to react with 300µL of the FRAP solution for 30 min in the dark condition. Readings of the colored product [ferrous tripyridyltriazine complex] were then taken at 593nm. The color of the reagent was changed from light orange to dark blue according to the percentage of the antioxidant present in the sample (Benzie and Strain 1999)

Total phenol content (TPC) of extracts was determined by Folin Ciocalteau method with some modifications. 20µL of the sample was added to 100µL 10% of Folin Ciocalteau reagent and placed for 5 minutes. 100µL of 1 g/ml of Na_2CO_3 were then added and the volume made up to 25 ml using distilled water. The solution was kept incubated at room temperature for 2 hours and Absorbance was measured at 517 nm. Gallic acid was used as standard and trolox, quercetin, Retin and ascorbic acid with different concentration as control groups.

DPPH (2, 2-Diphenyl-1-picrylhydrazyl) radical scavenging activity method was used to measure antioxidant capacity of food; where ethanol solution of DPPH (1mg/25ml) (200µl) was added to 10µl of extract solution with different concentrations (1 mg/ml). The DPPH solution was freshly prepared and kept in the dark at 4°C. Ethanol 96% (2.7 ml) was added and the mixture was shaken vigorously. The mixture was left to stand for 5 min and the absorbance was measured using a spectrophotometer at 517 nm. A blank sample containing the same amount of ethanol and DPPH was also

prepared. All determinations were performed in triplicate and the radical scavenging activities of the tested samples were expressed as percentage of inhibition according to the equation:

Percent of DPPH inhibition= [(AB-AA)/AB] × 100**;** Where AA and AB are the absorbance values of the test and of blank sample respectively and the percent inhibition versus concentrations was plotted as a curve.

3. RESULTS AND DISCUSSION

Table (1) The yield percentage of the extracts of *Bauhinia rufescens, Pongamia pinnata, Acacia sieberiana, Hardwickia binata* and *Prosopis juliflora* leaves

Name of plant	Solvent	Yield	Yield%	Solvent	yield	Yield%
B. rufescens	Petroleum ether	3.43	2.3	methanol	33.1	22.1
Pongamia pinnata	"	2.06	1.4	"	16.1	10.8
A. sieberiana	"	1.08	0.9	"	10.3	8.2
H. binata	"	3.33	2.2	"	46.58	31.9
Prosopis juliflora	"	3.79	2.5	"	23.9	23.9

In FRAP assay the ability of plant extract to reduce ferric ions was determined. The petroleum ether and methanol extracts were expressed as inhibition% measured at 00:00 minutes and after 00:04 minutes. The petroleum ether extract of all plants show high level of inhibition 52% to 80% after 4 minutes and; *H. binata* was constant in inhibition percentage of ferric reducing antioxidant power after 4 minutes (fig. 1). The methanol extract of

all plants show high ferric reducing antioxidant power ranging from 67.5% to 97 but *A. sieberiana* show less inhibition about 29% (Figs.1; 2 and 3).

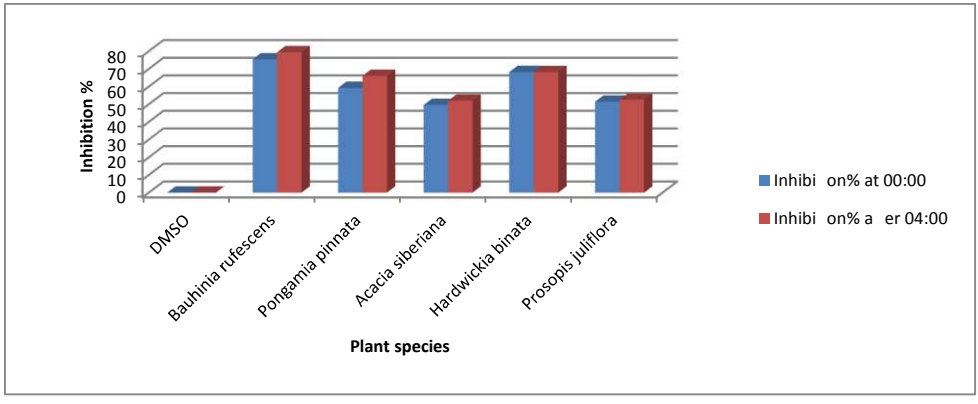

Fig (1) Ferric reducing antioxidant power of petroleum ether extracts of selected Sudanese medicinal plants leaves

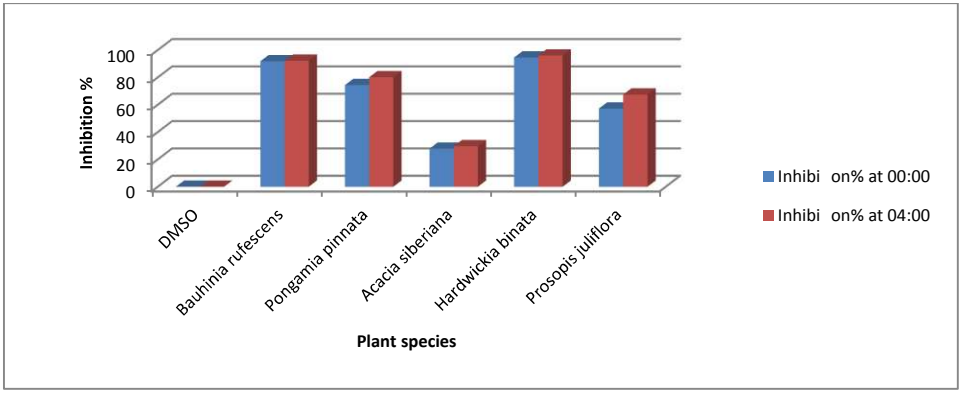

Fig (2) Ferric reducing antioxidant power of methanol extracts of selected Sudanese medicinal plants leaves.

Fig (3) Inhibition percentage of Ferric reducing antioxidant power of standards

The total phenol content in petroleum ether and 80% methanol extracts of 5 Sudanese medicinal plants was detected. The highest total phenol values were detected in the leaves of *H. binata and B. rufescens* ranging from (0.941-0.827µg/ml) respectively. While the extracts of *Pongamia pinnata, A. siberiana and Prosopis juliflora* were found containing few amount of total phenol ranging from (0.105-0.282µg/ml).The petroleum ether extract was found to have few amount of total phenol in all the plants (Fig. 4)

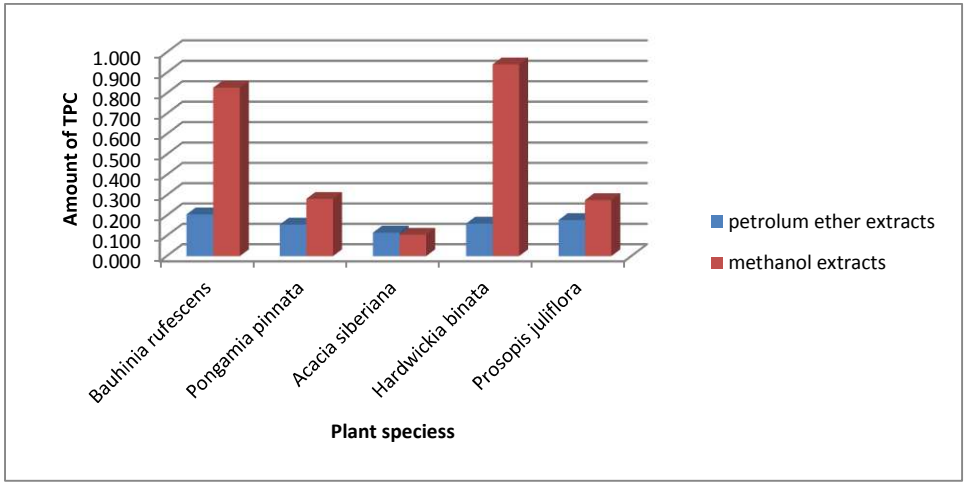

Fig (4) Total phenol content of petroleum ether and methanol extracts

DPPH: The free radical scavenging activity of the petroleum ether and methanol extracts were expressed as IC_{50} of inhibition**%** measured after each 40 minutes for the total of 120 minutes. The methanol extracts of *H. binata* and *B. rufescens* plant leaves showed very high scavenging activity against DPPH with IC_{50} (36.4 and 42.1 µg/ml) respectively compared to the standard Vitamin C (18.5µg/ ml) whereas; *Pongamia pinata* revealed moderate activity with IC50 (331.5µg/ ml). *A. sieberiana* and *Prosopis juliflora* showed low activity with IC_{50} ranging from (628.2-2018.9 µg/ml) respectively and this result was measured after 40 minutes. The activity of the plants was increased according to the increasing of time in all the plants (Fig. 5).

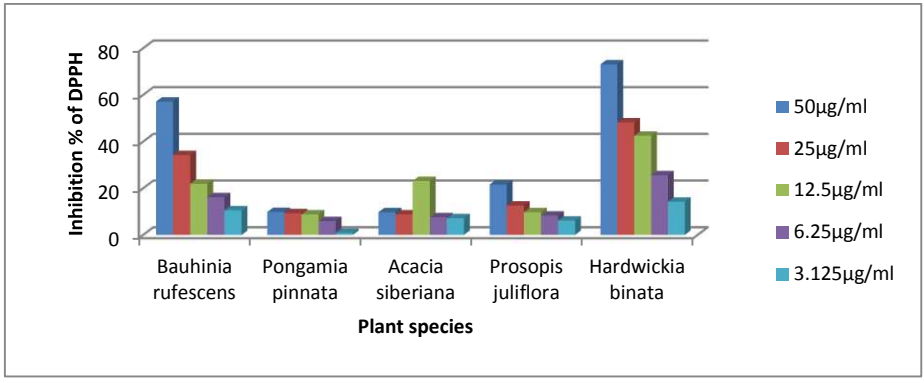

Fig (5) Inhibition percentage of free radical scavenging DPPH activity of the methanol extracts after 40 mint

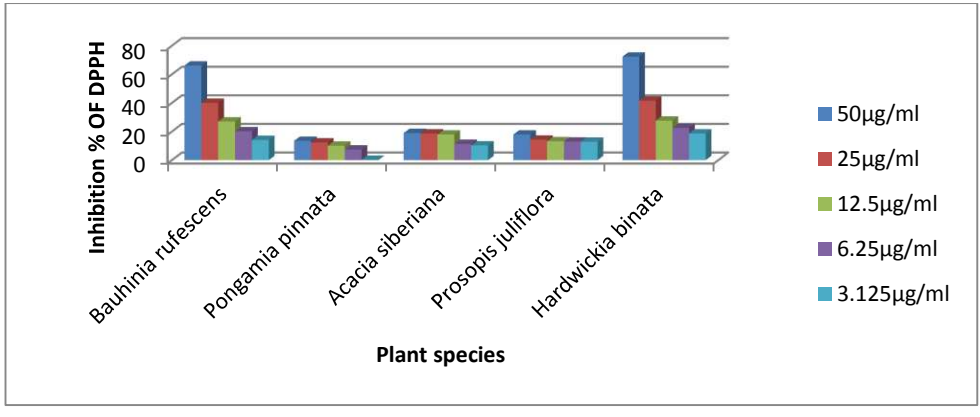

Fig (6) Inhibition percentage of free radical scavenging DPPH activity of the methanol extracts after 80 mint

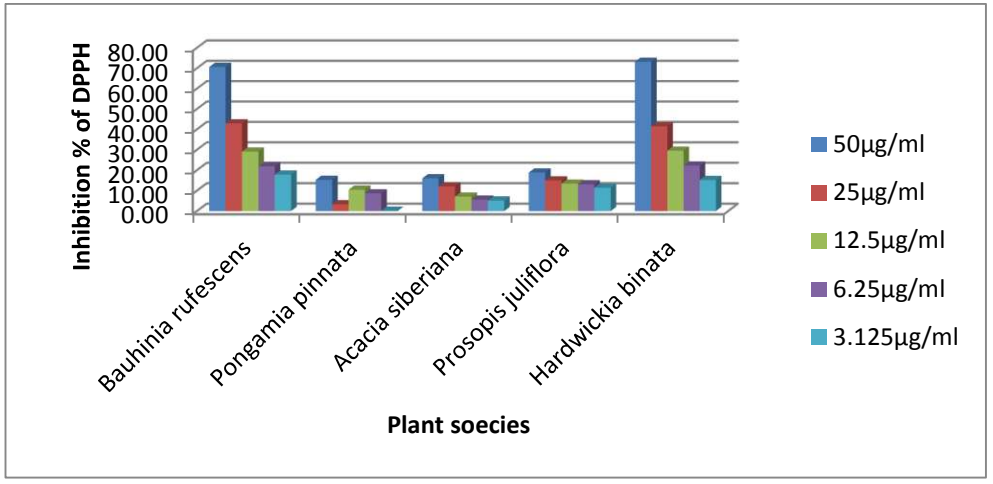

Fig (8) Inhibition percentage of free radical scavenging DPPH activity of methanol extracts after 120 mint

The petroleum ether extract of the plants *A. siberiana* and *B. rufescens* potent high scavenging activity of DPPH with IC_{50} (22.8-42.1 µg/ml) after 40 minutes and the extract of *Prosopis juliflora, Pongamia pinnata and H. binata* showed low activity with IC_{50} (566.3, 1785.1 and 1732.5µg/ ml).

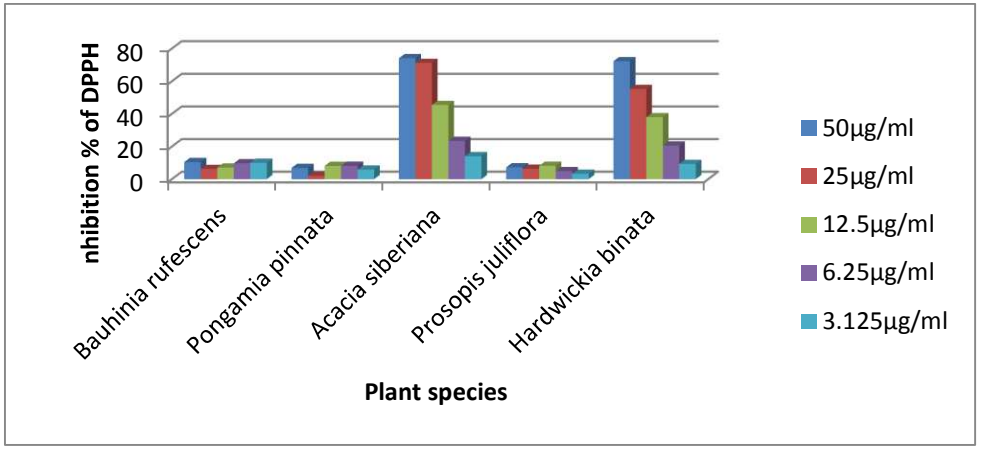

Fig (9) Inhibition percentage of free radical scavenging DPPH activity of petroleum ether extracts after 40 mint

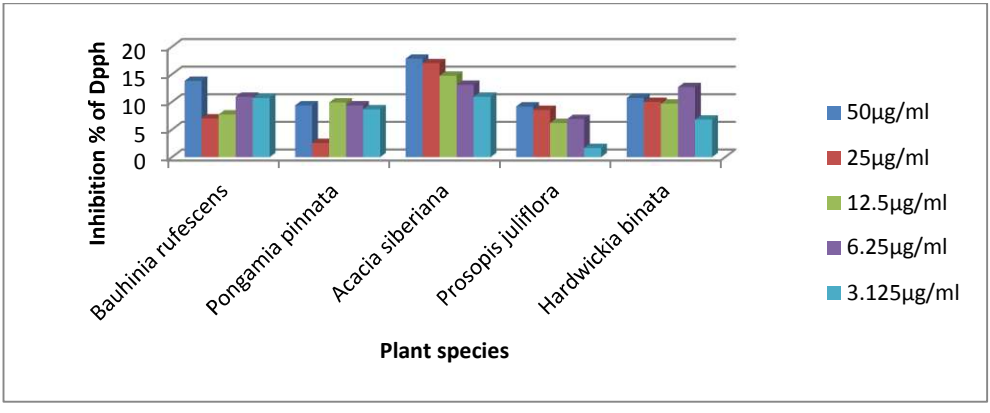

Fig (10) Inhibition percentage of free radical scavenging DPPH activity of petroleum ether extracts after 80 mint

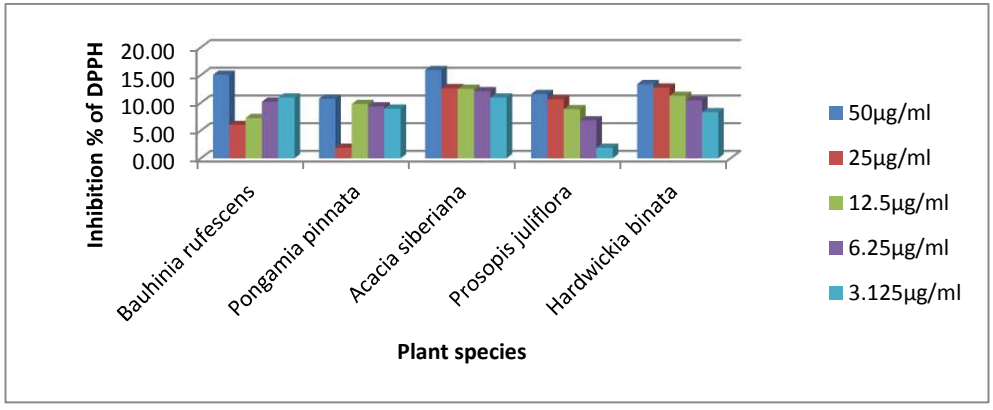

Fig (11) Inhibition percentage of free radical scavenging DPPH activity of petroleum ether extracts after 120 mint

The preliminary screening of anticancer activity for the five Sudanese selected Fabaceae plants against HePG2 cell line and MCF_7 showed that **(**Fig10**),** the petroleum ether extracts of the plants *B. rufescens, Pongamia pinnata*, *A. sieberiana* and *H. binata* showed high value of IC_{50} (13.8, 17.5, 18.0 and 28.7 µg/ml) respectively but only *Prosopis juliflora* showed low anticancer activity with IC_{50} (41.1 µg/ml). The methanol extract of the plant *Prosopis juliflora* observed to posses high anticancer activity against HepG2 with IC_{50} (10.0 µg/ml) followed by the plants *B. rufescens* and *Acacia sieberiana* and *P. pinnata* with IC_{50} (26.0, 27.8 and 29.5 µg/ml) respectively but *H. binata* showed low activity with IC50 (44.90 µg/ml).

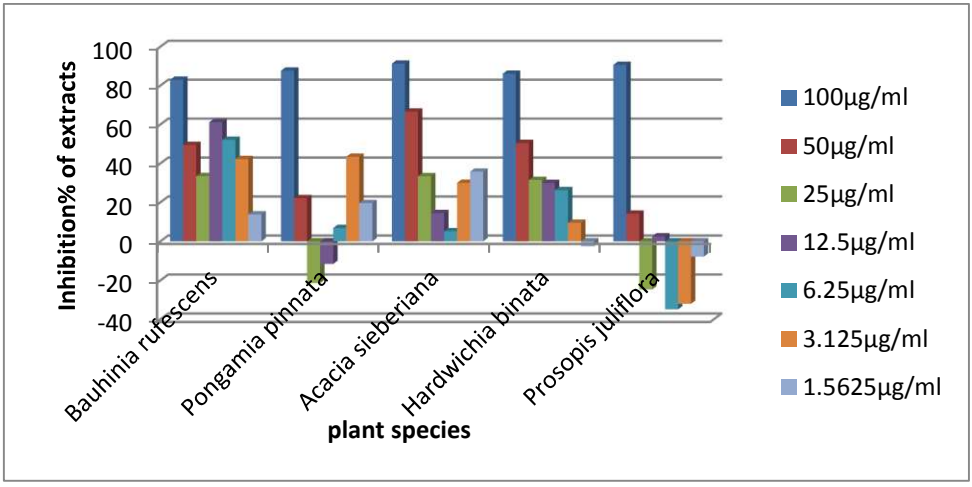

Fig (12) Inhibition% of petroleum ether extracts against He PG 2cell line using MTT assay

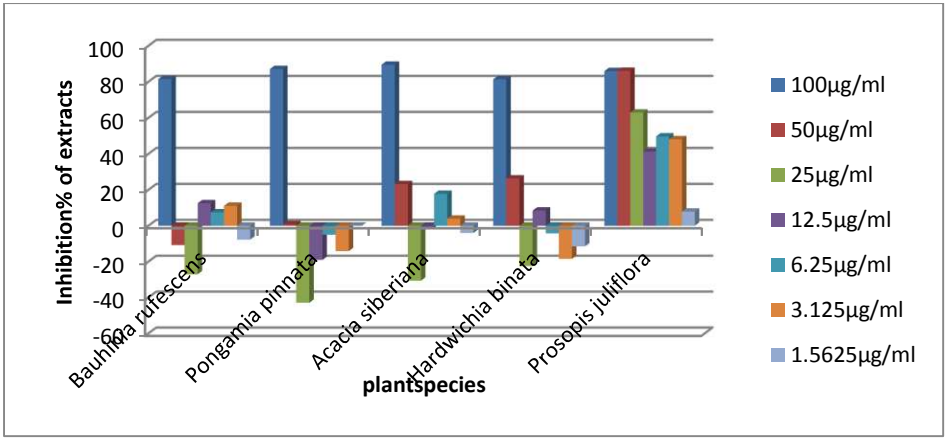

Fig (13) Inhibition% of methanol extracts against He PG 2cell line using MTT assay

The anticancer activity of the petroleum ether extract against the cell line MCF7 revealed highest values of IC_{50} (3.8, 6.8, 7.3, 14.2 and 20.7 µg/ml) respectively in all plants *Pongamia pinnata, Prosopis juliflora, B. rufescens, H. binata* and *A. sieberiana*. The methanol extract of the plants *Prosopis juliflora* and *Pongamia pinnata* showed high anticancer activity against MCF7 with IC_{50} (14.9 and 17.3 µg/ml) respectively. While the plants *A. sieberiana, H.binata, B. rufescens* revealed low activity with IC_{50} (30.9, 30.9 and 39.0 µg/ml) respectively.

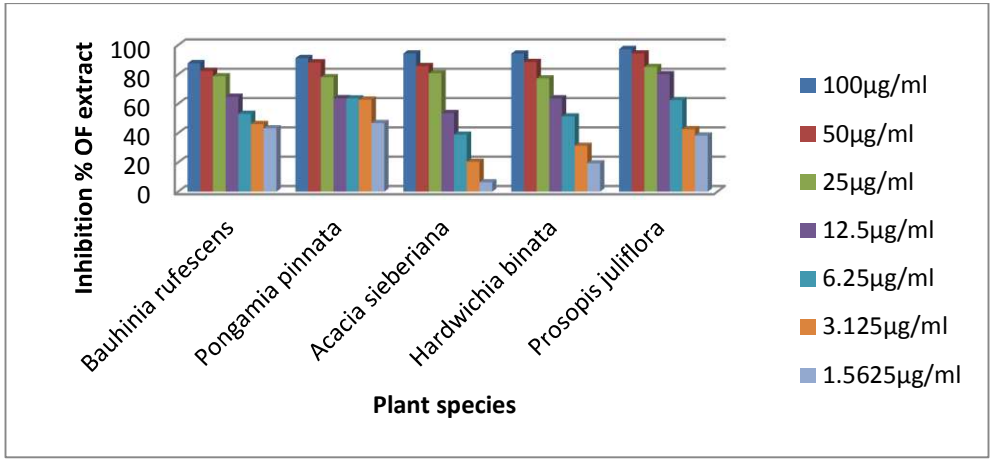

Fig (14) Inhibition percentage of petroleum ether extracts against MCF7cell line using MTT assay

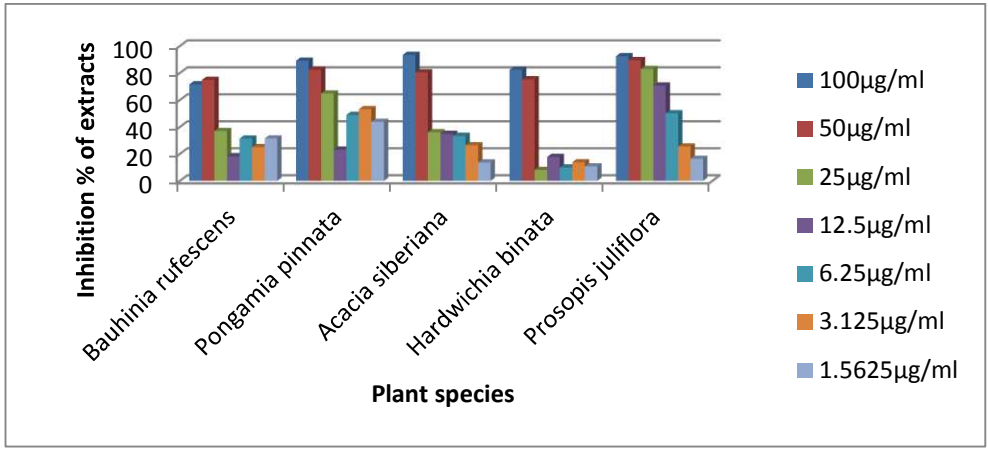

Fig (15) Inhibition percentage of methanol extracts against MCF7cell line using MTT assay

Cancer becomes the second leading cause of death in the world despite the fact that; breast cancer has been reported to be the leading malignancy according to estimate data from GLOBOCAN. However studies on breast cancer in Sudan have been limited due to; lack of population based cancer registry as well as lack of research resources (Elgaili *et al*., 2010). For ages plants have been a good source of food and they provide essential nutritional values, medicinal properties and notable physiological effect to life (Mouli *et al.*, 2009). As source of medicines, plants have formed the basis for sophisticated traditional systems and continue providing mankind with new remedies. In recent years, the interest in folk medicine has highly increased (Sara *et al.*, 2009).

The antioxidants may prevent and cure cancer and other diseases by protecting cells from damaging caused by free radicals, the highly reactive oxygen compounds. Many naturally occurring substances present in human diet have been identified as potential chemo-preventive agents; and consuming relatively large amounts of vegetables and fruits that can prevent the development of cancer. In this study five plants of the family Fabaceae was investigated for their anticancer by using two cell line HepG2 (Liver cancer) and MCF7 (Breast cancer).The antioxidant activity was determined by using FRAP, DPPH AND TPC. The methanol extract of all the plant accept *Hardwickia binata* showed high anticancer activity against MCF_7 and $HepG_2$ The study showed that *Prosopis juliflora* contain many secondary metabolites compounds where the leaves contain tannins, acids, glycosides, flavonoids and alkaloids by (Sathiya and Muthuchelian ,2008) and that may explain the high activity of methanolic extracts of this plant against HePG2 and MCF7 cell line .The plant *Pongamia pinnata* used in folk medicine for treatment of tumor and various diseases explains its high activity against HePG2 and MCF7 cell lines. 113 cell line with IC_{50} >30µg/ml. The methanol extract of *Prosopis juliflora* revealed the most active result with IC50 (9.96). Similarly the work of Sathiya and Muthuchelian (2008) in the leaves of *Prosopis juliflora* revealed the presence of tannins, acids, glycosides, flavonids and alkaloids. Hence the presence of alkaloids might explain the anticancer activity of this plants and the presence of flavonoids that might be the reason of antioxidant activity which have a role in anticancer activity of

medicinal plants. The ethanol extracts of the leaves of *Hardwickia binata* was found by the work of Santosh *et al*. (2007) to contain phenolics flavonoids, saponins and tannins and similarly; the ethanol extracts of *B. rufescens* contain phenolics flavonoids, saponins and tannins. Thus the agreements that were obtained from the phytochemical screening of the plants tested explain their anticancer and antioxidants activity.

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