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THE *IN VITRO* EVALUATION OF ANTIOXIDANT, ANTICANCER AND ANTIMICROBIAL PROPERTIES OF *ARAUCARIA HETEROPHYLLA* GROWN IN EGYPT

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Abstract

In order to validate *Araucaria heterophylla* antioxidants, anticancer and antimicrobial properties with respect to traditional uses, we have screened for the first time the activity of aerial parts of the plant against different pathogenic microorganisms and the cytotoxic activity against different cell lines. It has revealed high total phenolic content values ranged from 383.65 to 70.0 mg GAE/g extract for the plant extracts. The total antioxidant capacity (TAC) of different solvent extracts using phosphomolybdenum antioxidant assay revealed the most active *n*-BuOH extract among the tested extracts was 506.66 mg AAE /g dry extract. Moreover the reducing power assay supported the results of phosphomolybdenum assay that, the *n*-BuOH extract showed high reducing power activity (OD value) with 0.860 compared to ascorbic acid (0.905). The *in vitro* anticancer activity of different solvent extracts of *A. heterophylla* was evaluated against four human tumor cells namely; Hepatocellular carcinoma (HEPG-2), Mammary gland breast cancer (MCF-7), Epitheliod Carcinoma (Hela) and Human prostate cancer (PC-3). For HePG-2 cell line, the IC_{50} values for the tested extracts ranged from 10.62 to 34.21 μg/ ml compared to Doxorubicin with IC_{50} value equal 4.50 μg/ml. While, for MCF-7 cell line, the IC_{50} values for the tested extracts ranged from 7.64 to 34.30 μg/ ml compared to Doxorubicin with IC_{50} equal to 4.17 μg/ml. Moreover, for PC3 cell line, the IC_{50} values for the tested extracts ranged from 15.39 to 54.90 μg/ ml compared to Doxorubicin with IC_{50} equal to 8.87 μg/ml. While, for Hela cell line, the IC_{50} values for the tested extracts ranged from 6.72 to 27.78 μg/ ml compared to Doxorubicin with IC_{50} equal to 5.57 μg/ml. The antimicrobial activity was evaluated via cup agar method using four pathogenic bacterial & fungal strains e.g. *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans* and, *Aspergillus niger*. We conclude that, this investigation showed that *A. heterophylla* has a potent antioxidant, anticancer and antimicrobial activities, so it confirm the use of this plant as medicinal agent. Further research is required to evaluate the practical values of therapeutic applications.

Keywords: *antioxidants, anticancer, Araucaria heterophylla, extracts, antimicrobial*

Introduction

Natural products from plants and herbs have been used extensively as alternative drugs for the treatment of a wide range of ailments and diseases [1,2]. Most of the well-known diseases are caused by oxidative stress occurring within the body as a result of chemical exposure or pathogen infection, whereby the affected organism cannot produce sufficient antioxidants to overcome the produced free radical, also known as reactive oxygen species. Therefore, the body needs an exogenous source of antioxidants. Plants or their byproducts are thus preferred because of their richness with polyphenolics, alkaloids, quinones, volatile oil and terpenoids that proved to be effective alternatives to many synthetic compounds [3-6]. Reactive oxygen species (ROS) are highly unstable compounds containing an odd electron has the ability to attack cells and tissues in the human body [7]. Moreover, the accumulation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the human body can cause oxidative stress, which is associated with several health disorders such as cancer, inflammation, neurodegeneration, and cardiovascular diseases [8-11]. Cancer defined as a rapid abnormal uncoordinated proliferation of aberrant cells in tissue or organ of the human body which may mass together to form tumor. Otherwise, cancer is considered as one of the most fearsome causes of morbidity and mortality in all over the world after heart disease, and must be fight via surgery or chemotherapy [7,12-14]. Recently, the resistance of the pathogenic microbial strains against antibiotics develops much faster than ever. Infectious diseases caused by bacterial and fungal infections are still a major threat to public health, despite the tremendous progress in human medicine. The past three decades have seen a dramatic increase in microbial resistance to antimicrobial agents. Such situation stimulates the development of new antimicrobial agents to treat the infectious disease in an effective manner. So, this matter continued to an era to identify the potential antimicrobial agent from the natural resources [15,16]. Our plant is *Araucaria heterophylla* while, Araucaria: A genus of evergreen coniferous trees in the family Araucariaceae [17]. *A. angustifolia* showed to be has a Phenolic acids, flavonoids and lignans [18,19]. In view of the wide continued interest in the biological activity profile of this important herb grown in Egypt, the current study reports also its anticancer, antioxidant and antimicrobial effects.

Materials for biological studies

Chemicals for biological studies

I- Phosphomolybdenum assay (TAC)
Sodium phosphate monobasic (Merck/ Germany), ammonium molybdate [$(NH_4)_6$ $Mo_7O_{24}.4H_2O$] (El-Nasr Pharmaceutical Chemicals Co. /Egypt), ascorbic acid (Merck Chemical Co.) and sulphuric acid (Sigma-Aldrich Co.).

II- Reducing Power Antioxidant Assay (RPAA)
Trichloroacetic acid (TCA) (Merck Chemical Co.), ferric chloride (Merck Chemical Co.), potassium ferricyanide [K_3 Fe $(CN)_6$] (Merck Chemical Co.) and [buffer solution components pH 6.6; NaCl (ADWIC Co. / Egypt), KCl (Merck/ Germany), Na_2HPO_4 (Merck/ Germany) and KH_2PO_4 (ADWIC Co. / Egypt)] and ascorbic acid (Merck Chemical Co.).

III- Total phenolic Content (TPC)
Folin-Ciocalteu's phenol reagent (Sigma-Aldrich Co.), sodium carbonate (Na_2CO_3; Merck / Germany) and gallic acid (Merck Chemical Co.).

Polyphenols (Flavonoids & Phenolic acids)

Gallic acid, quercetin, kaempferol, luteolin, apigenin, chlorogenic acid, taxifolin, rutin were obtained from Medicinal Chemistry Department, Theodor Bilharz Research Institute Giza, Egypt.

Materials and Methods

Plant material

The fresh leaves of *Araucaria heterophylla* were collected from Prince Mohamed Ali Palace, Cairo, during March (2014). The identity of the plant was established by Dr. Threase Labib, Consultant in Orman Botanical Garden and National Gene Bank. The plant material was air-dried and kept in tightly closed container.

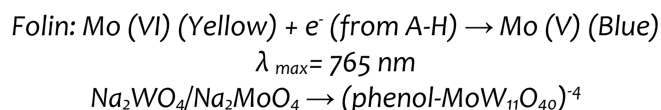
Extraction and Fractionation

The air-dried powdered leaves of *A. heterophylla* (2.5 kg) were extracted with 85% methanol at room temperature (25 ± 2°C) for several times (3 liter x 5 times). The 85% methanolic extract was concentrated

under reduced vacuum using rotatory evaporator (40 ± 2°C) to yield 270 g, then 260 g from 85% methanolic extract was defatted with petroleum ether (60-80°C) to give petroleum ether fraction 25 g. The defatted 85% methanol extract (235 g) was fractionated using CH_2Cl_2, EtOAc, and *n*-BuOH. The obtained fractions were concentrated to give 45, 55, 70, respectively and 60 g free sugar (H_2O extract).

Determination of Total Phenolic Content (TPC)

The total phenolic content of each plant extract was determined using Folin - Ciocalteu's reagent according to the reported method [20], gallic acid was used as standard. In this method, the reaction mixture was composed of (100 µl) of plant extract (100 µg/ml), 500 µl of the Folin-Ciocalteu's reagent and 1.5 ml of sodium carbonate (20%). The mixture was shaken and made up to 10 ml using distilled water. The mixture was allowed to stand for 2 hrs. Then the absorbance was measured at 765 nm using spectrophotometer (UV-VS spectrophotometer, Milton Roy 601, Co, USA). All determinations were carried out in triplicate. The total phenolic content was expressed as mg gallic acid equivalent (GAE) per g extract. This method is based on an oxidation/reduction reaction of a molybdotungstate reagent. The electron transfer reaction leads to the formation of the blue color, which can be simply quantified by spectrophotometry at 765 nm. Folin: molybdophosphotungstate heteropolyanion reagent, in which Mo (VI) is reduced to Mo (V) with an electron donated by an antioxidant as follows [21].



Folin: Mo (VI) (Yellow) + e^- *(from A-H) → Mo (V) (Blue)*

$$\lambda_{max} = 765\ nm$$

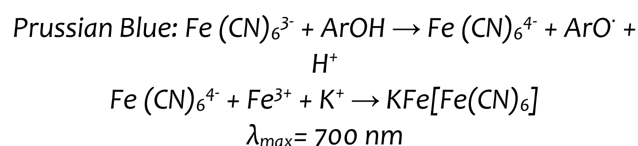
$$Na_2WO_4/Na_2MoO_4 \rightarrow (phenol\text{-}MoW_{11}O_{40})^{-4}$$

Determination of Total Antioxidant Capacity (TAC)

The antioxidant activity (AOA) of each sample (extract, fraction or pure compound) was determined according to phosphomolybdenum method using ascorbic acid as standard. This assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of a green colored [phosphate=Mo (V)] complex at acidic pH. In this method, 0.5 ml of each extract (100 µg /ml) in methanol was combined in dried vials with 5 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The vials containing the reaction mixture were capped and incubated in a thermal block at 95°C for 90 min. After the samples had cooled at room temperature, the absorbance was measured at 695 nm against a blank. The blank consisted of all reagents and solvents without the sample and it was incubated under the same conditions. All experiments were carried out in triplicate. The antioxidant activity of the sample was expressed as the number of equivalents of ascorbic acid (AAE) [22].

Reducing Power Antioxidant Assay (RPAA)

The spectrophotometric method described by Ferreira *et al.*, 2007; was used for the measurement of reducing power. For this 2.5 ml of each of the sample (extract, fraction or pure compound) was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide (10 mg/ml). The mixture was incubated at 50°C for 20 min, then rapidly cooled, mixed with 2.5 ml of 10% trichloroacetic acid and centrifuged at 6500 rpm for 10 min. An aliquot (2.5 ml) of the supernatant was diluted with distilled water (2.5 ml) and then ferric chloride (0.5 ml, 0.1%) was added and allowed to stand for 10 min. The absorbance was read spectrophotometrically at 700 nm, ascorbic acid used as standard. Three replicates were made for each test sample. This method is based on the ability of the antioxidant molecules, which have reduction potential, to react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form blue coloured ferric ferrous complex $(Fe^{3+})_4[Fe^{2+}(CN^-)_6]_3$ that has an absorption maximum at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power of the sample, reducing power was reported as ascorbic acid equivalent per gm of dry sample [23].



Prussian Blue: $Fe(CN)_6^{3-} + ArOH \rightarrow Fe(CN)_6^{4-} + ArO^{\cdot} + H^+$

$$Fe(CN)_6^{4-} + Fe^{3+} + K^+ \rightarrow KFe[Fe(CN)_6]$$

$$\lambda_{max} = 700\ nm$$

Antimicrobial activity by cup plate method

The samples were prepared by dissolving samples in methanol (0.025 g in 5ml methanol) and 100µl was used in this test. The antimicrobial activity of different samples was investigated by the agar cup

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plate method. Six different test microbes namely: *Staphylococcus aureus* (G+ve), *Pseudomonas aeruginosa* (G-ve), *Candida albicans* (yeast), *Aspergillus niger* (fungus), *Trichoderma viride* and *Chaetomim globodum* were used. The bacterial and yeast test microbes were grown on a nutrient agar medium (DSMZ1) of the following components (g/l): Peptone (5.0), Meat extract (3.0), Agar (20.0), distilled water (1000.0 ml) and the pH to 7.0. On the other hand, the fungal test microbe was cultivated on Potato-Dextrose agar (PDA) medium (DSMZ129) of the following ingredients (g/l): Infusion from 200g potatoes, glucose (20), distilled water (1000.0ml) and the pH was recorded to be 6. 100µl of stock culture from bacteria (107 to 108CFU) and fungi (106 to 107CFU) were spreaded on the top of agar plates containing the proper medium using sterilized cotton swab. Then holes (1cm diameter) were made in media by gel cutter (Cork borer) in sterile condition. Then one drop of melted agar was poured into hole and allowed to solidify to make a base layer. After that specific amount of tested sample (0.1 ml) was poured into the hole. Then plates were kept at low temperature (4°C) for 2-4 hours to allow maximum diffusion. The plates were then incubated at 37°C for 24 hours for bacteria and at 30°C for 48 hours in upright position to allow maximum growth of the organisms. The antimicrobial activity of the test agent was determined by measuring the diameter of zone of inhibition expressed in millimeter (mm). The experiment was carried out more than once and mean of reading was recorded [24,25].

MTT assay

The cell lines mentioned above were used to determine the inhibitory effects of compounds on cell growth using the MTT assay. This colorimetric assay is based on the conversion of the yellow tetrazolium bromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells. Cell lines were cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics added were 100 units/ml penicillin and 100µg/ml streptomycin at 37°C in a 5% CO_2 incubator. The cell lines were seeds in a 96-well plate at a density of $1.0x10^4$ cells/well at 37°C for 48 h under 5% CO_2. After incubation the cells were treated with different concentration of compounds and incubated for 24 h. After 24 h of drug treatment, 20 µl of MTT solution at 5mg/ml was added and incubated for 4 h. Dimethyl sulfoxide (DMSO) in volume of 100 µl is added into each well to dissolve the purple formazan formed. The colorimetric assay is measured and recorded at absorbance of 570 nm using a plate reader (EXL 800, USA). The relative cell viability in percentage was calculated as (A570 of treated samples/A570 of untreated sample) X 100 [26,27].

Statistical Analysis

All data were presented as mean ± S.D. using SPSS 13.0 program. Correlation analysis of the antioxidant activity and free radical scavenging activity versus the total phenolic content of the different extracts of tested plant were carried out using the correlation and regression by Microsoft Excel program.

Results and discussion

Total phenolic content (TPC)

Different solvent extracts of *A. heterophylla* were evaluated for their TPCs. The results revealed that *n*-BuOH extract showed the highest content of polyphenols with 383.65 mg GAE/g extract, followed by 278.33, 255.0, 138.35 and 70.0 mg GAE/g extract for 85% methanol, EtOAc, petroleum ether and dichloromethane extracts, respectively (Table 1).

The total phenolic assay is routinely used because it is simple, sensitive and precise and actually measures the reducing capacity of the tested samples and their antioxidant activities have been attributed to their phenolic contents [21,28]. Folin-Ciocalteu's reagent (FCR), exist as molybdophosphotungstate heteropolyanion (3 H_2O-P_2O_5-13WO_3-5 MoO_3-10H_2O), in which Mo(VI) is reduced to Mo(V) with an electron donated by an antioxidant. Under alkaline conditions, Folin–Ciocalteu's reagent (yellow color) reacts with phenolic compounds and, consequently, a phenolate anion is formed by dissociation of a phenolic hydrogen atom. This sequence of reversible one- or two-electron reduction reactions leads to blue-coloured chromophores being formed between phenolate and the FCR reagent [21].

Biological activities

Plants are an excellent source of chemical compounds with a wide variety of biological activities, including anticancer properties.

Fundamental phytochemical investigations should be encouraged, especially in view of the urgent need to discover new bioactive molecules with greater efficacy and less side effects than existing drugs [29,30].

Total antioxidant capacity (TAC)

The total antioxidant capacity (TAC) of the tested extracts was evaluated via phosphomolybdenum antioxidant assay. This assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of a green colored [phosphate=Mo (V)] complex at acidic pH with a maximal absorption at 695 nm [22,31,32]. In this assay, the (TAC) value of the most active *n*-BuOH extract was 506.66 mg AAE /g dry extract, followed by 448.20, 370.25, 397.94 and 248.20 mg AAE /g dry extract 85% methanol, EtOAc, petroleum ether and dichloromethane extracts, respectively (Table 2).

Polyphenolic compounds such as flavonoids, phenolic acids and tannins are considered to be the major contributors to the antioxidant activity of medicinal plants, fruits and vegetables. The antioxidant activities of polyphenols were attributed to their redox properties, which allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers, as well as their metal chelating abilities [10,33,34].

Reducing power antioxidant activity (RPAA)

Antioxidative properties of polyphenols arise from their high reactivity as hydrogen or electron donors from the ability of the polyphenol derived radical (Ar-O·) to stabilize and delocalize the unpaired electron (chain-breaking function) and from their potential to chelate metal ions (termination of the Fenton reaction; $Cu^{+}/Fe^{2+} + H_2O_2 \rightarrow Cu^{2+}/Fe^{3+} + HO^{-} + HO^{\bullet}$) [35,36]. Reducing power assay measure the ability of tested samples to transform the ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) via electron donation [37]. Reducing power assay supported the results of phosphomolybdenum assay. Moreover, in this assay, the *n*-BuOH extract showed high reducing power activity (OD value) with 0.860, followed by EtOAc (0.787), MeOH (0.540), petroleum ether (0.465) and CH_2Cl_2 (0.392), respectively, compared to ascorbic acid (0.905) (Figure 1).

The reducing properties are generally associated with the presence of reductones, which have been shown to exhibit antioxidant action by breaking the chain reactions and donating a hydrogen atom. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation [38]. Being good electron donors, phenolic compounds show the reducing power and have ability to convert the ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) by donating an electron [39]. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [40].

In vitro anticancer activity

Cancer as malignant disease is one of the major causes of death in humans [41]. It is well known that cancer is second only to cardiovascular disease as a natural cause of death, with an incidence of over 6 million cases reported annually across the globe [42]. Thus, it is urgent to find more and safer new active constituents that attack and kill cancer cells.

Phenolic compounds have vital role on cancer chemoprevention and chemotherapy. Additionally, many mechanisms and modes of action have been identified, involving carcinogen inactivation, ant proliferation, cell cycle arrest, induction of apoptosis and differentiation, inhibition of angiogenesis and ant oxidation or a combination of these mechanisms [43]. Naturally occurring antioxidant compounds have a vital role in cancer prevention and treatment [44].

Therefore, the current study was directed to the exploration of the *in vitro* anticancer activity of different solvent extracts of *A. heterophylla* against four human tumor cells namely; Hepatocellular carcinoma (HEPG-2), Mammary gland breast cancer (MCF-7), Epitheliod Carcinoma (Hela) and Human prostate cancer (PC-3). Results are presented in Tables 3-9 and Figures 2-7.

For HePG-2 cell line, the IC_{50} values for the tested extracts ranged from 10.62 to 34.21 µg/ ml compared to Doxorubicin with IC_{50} equal to 4.50 µg/ml. The results are in the order: EtOAc > BuOH > H_2O > CH_2Cl_2 > pet. ether (Table 3).

For MCF-7 cell line, the IC_{50} values for the tested extracts ranged from 7.64 to 34.30 µg/ ml compared to Doxorubicin with IC_{50} equal to 4.17 µg/ml. The results are in the order: EtOAc > BuOH > H_2O > Pet. ether > CH_2Cl_2 (Table 3).

For PC3 cell line, the IC_{50} values for the tested extracts ranged from 15.39 to 54.90 µg/ ml compared

to Doxorubicin with IC_{50} equal to 8.87 µg/ml. The results are in the order: EtOAc > BuOH > H_2O > Pet. ether > CH_2Cl_2 (Table 3). While, for Hela cell line, the IC_{50} values for the tested extracts ranged from 6.72 to 27.78 µg/ ml compared to Doxorubicin with IC_{50} equal to 5.57 µg/ml. The results are in the order: EtOAc > BuOH > H_2O > Pet. ether > CH_2Cl_2 (Table 3).

Abdel-Sattar *et al* (2009) reported that the resin extract of *A. heterophylla* growing in Egypt exhibited a strong cytotoxic activity against breast (MCF7) and colon (HCT116) cancer cell lines with IC_{50} values of 0.54 and 0.94 µg/ml, respectively [45].

Antimicrobial activity by cup plate method

The antimicrobial activity was evaluated via cup agar (100 microliter per cup) method using four pathogenic bacterial & fungal strains e.g. *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans* and , *Aspergillus niger*. The results in Table (10) and Figure (8) showed that the tested extracts exhibited antimicrobial activity against the four strains expressed by inhibition zones which ranged from 0 to 23mm (*S. aureus*), from 0 to 22mm (*P. aeruginosa*), from 0 to 22mm (*C. albicans*) and from 11 to 14 mm (*A. niger*). The different extracts of *A. heterophylla* leaves showed strong antimicrobial activity, which may be return to the synergistic action (Co-activity) of their chemical constituents especially flavonoids and phenolic acids [56].

Conclusion

The n-butanol extract of *A. heterophylla* leaves showed a high antioxidant, antimicrobial and anticancer activities due to it is rich in flavonoids and phenolic content, which can be formulated in many important phytopharmaceutical preparations containing the standardized bioactive ingredient. Development of methods for standardization of extracts prepared from the plant and study of their stability and bioavailability. Using of plant extracts as a raw material in the manufacture of some biopharmaceuticals, which could contribute to the treatment of microbial diseases in Egypt, and this will be after application of pharmaceutical pharmacy standards and toxicity study for the using of natural product in the manufacture of biopharmaceutical preparations. Clinical trials should be done to support the previous investigations and to facilitate the production of new drugs in the Egyptian market.

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Table 1. Total phenolic content (TPC) of different solvent extracts of *A. Heterophylla*

Sample	Total phenolic (mg gallic acid equivalent/ g dry extract)[1]
85% MeOH	278.33 ± 5.77
Pet. ether	138.35 ± 7.63
CH_2Cl_2	70.0 ± 5.0
EtOAc	255.0 ± 5.0
n-BuOH	383.65 ± 7.0

Results are expressed as mean values ± standard deviation (n = 3).
[1]TPC (total phenolic content) values are expressed as mg gallic acid equivalent/g extract (mg GAE/g ext.).

Table 2. Total antioxidant capacity (TAC) of different solvent extracts of *A. Heterophylla*

Sample	Total antioxidant capacity (mg AAE /g dry ext.)[1]
85% MeOH	448.20 ± 3.55
Pet. ether	397.94 ± 1.77
CH_2Cl_2	248.20 ± 3.50
EtOAc	370.25 ± 1.72
n-BuOH	506.66 ± 1.65

[1]Total antioxidant capacity (TAC) monitored by the phosphomolybdenum assay expressed as mg ascorbic acid equivalent AAE /g dry extract.

Table 3. Anticancer activity of different solvent extracts of *A. heterophylla* against four human tumor cells

Sample	***In vitro*** **anticancer (IC_{50} µg/ ml)[1]**			
	HePG-2	**MCF-7**	**PC3**	**Hela**
DOX[2]	4.50±0.2	4.17±0.2	8.87±0.6	5.57±0.4
H_2O	16.86±1.5	23.76±1.9	20.99±1.8	9.84±1.0
EtOAc	10.62±1.1	7.64±0.8	15.39±1.3	6.72±0.5
Pet. ether	34.21±2.3	31.93±2.2	38.09±2.4	19.34±1.8
BuOH	12.06±1.4	9.13±1.0	17.42±1.5	7.69±0.9
CH_2Cl_2	26.49±2.1	34.30±2.5	54.90±2.9	27.78±2.6

[1]IC_{50} (µg/ml): 1-10 (very strong). 11-20 (strong). 21-50 (moderate). 51-100 (weak) and above 100 (non-cytotoxic).
[2]DOX: Doxorubicin.

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Table 4. Average of Relative viability of cells (%) of DOX.

Conc. (µg/ml)	HePG-2	MCF-7	PC-3	Hela
DOX				
100	6.3	6.2	8.8	7.3
50	11.2	10.9	16.3	12.1
25	14.1	14.3	21.7	18.9
12.5	28.3	26.9	38.9	30.8
6.25	45.8	41.5	59.2	51.7
3.125	57.6	58.4	73.6	62.4
1.56	71.2	69.1	95.3	74.0

Table 5. Average of Relative viability of cells (%) of H_2O.

Conc. (µg/ml)	HePG-2	MCF-7	PC-3	Hela
H_2O				
100	20.9	24.3	23.7	12.7
50	32.7	36.1	34.3	19.3
25	39.1	47.2	42.8	27.1
12.5	50.2	59.4	56.1	38.2
6.25	64.4	73.2	70.4	61.5
3.125	87.5	91.6	93.6	74.4
1.56	100	100	100	97.1

Table 6. Average of Relative viability of cells (%) of EtOAc.

Conc. (µg/ml)	HePG-2	MCF-7	PC-3	Hela
EtOAc				
100	13.7	7.8	18.1	7.3
50	21.3	16.3	25.7	13.7
25	27.1	23.9	38.2	19.4
12.5	42.6	33.7	46.3	36.1
6.25	61.5	56.0	69.4	52.8
3.125	75.4	68.1	90.1	63.5
1.56	98.2	89.2	100	85.2

Table 7. Average of Relative viability of cells (%) of Pet. ether.

Conc. (µg/ml)	HePG-2	MCF-7	PC-3	Hela
Pet. ether				
100	29.6	31.3	32.7	23.5
50	41.7	43.7	42.9	30.7
25	52.3	50.9	57.1	41.3
12.5	71.8	62.6	68.3	56.1
6.25	83.5	77.2	89.2	69.4
3.125	98.2	96.1	100	90.0
1.56	100	100	100	100

Table 8. Average of Relative viability of cells (%) of BuOH.

Conc. (µg/ml)	HePG-2	MCF-7	PC-3	Hela
BuOH				
100	15.6	7.5	20.5	8.4
50	26.1	17.3	27.1	18.6
25	34.3	26.1	41.9	22.5
12.5	41.9	35.8	48.4	37.1
6.25	60.3	56.7	70.3	51.3
3.125	78.4	81.3	95.7	70.4
1.56	100	93.9	100	89.1

Table 9. Average of Relative viability of cells (%) of CH_2Cl_2.

Conc. (µg/ml)	HePG-2	MCF-7	PC-3	Hela
CH_2Cl_2				
100	24.8	30.4	40.3	26.8
50	37.1	41.7	51.4	39.1
25	49.0	53.9	63.7	51.2
12.5	61.2	68.1	75.0	59.4
6.25	80.3	84.2	93.5	78.3
3.125	95.9	97.3	100	96.1
1.56	100	100	100	100

Table 10. Antimicrobial activity (inhibition zones) of the different solvent extracts of *A. heterophylla* leaves

Sample	Clear zone (ϕmm)			
	Staphylococcus aureus	*Pseudomonas aeruginosa*	*Candida albicans*	*Aspergillus niger*
1- CH_2Cl_2	16	22	20	11
2- Pet. ether	23	19	20	11
3- *n*-BuOH	17	19	16	13
4- H_2O	0	0	0	12
5- EtOAc	17	21	22	14

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Fig. 1. Reducing power antioxidant activity (RPAA) of different solvent extracts of *A. heterophylla* at concentration 200 µg/ml against ascorbic acid.

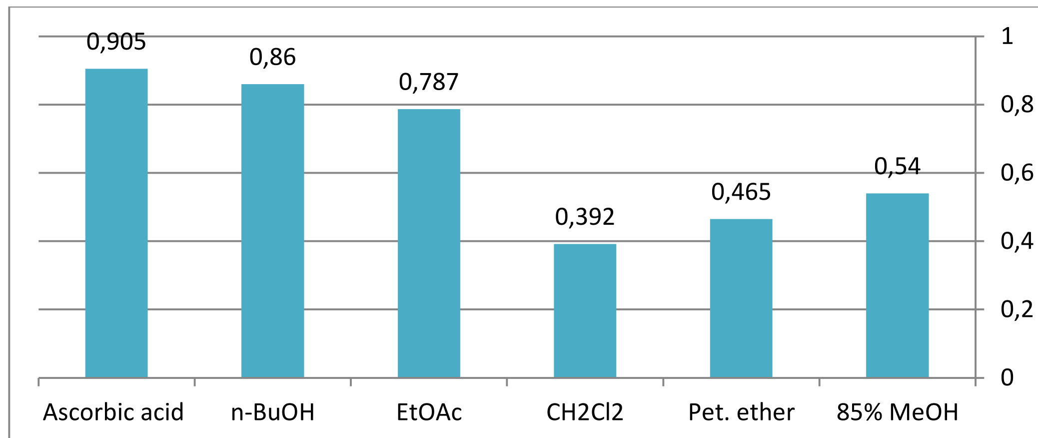

Fig. 2. Relative viability cells (%) using different concentrations of Doxorubicin.

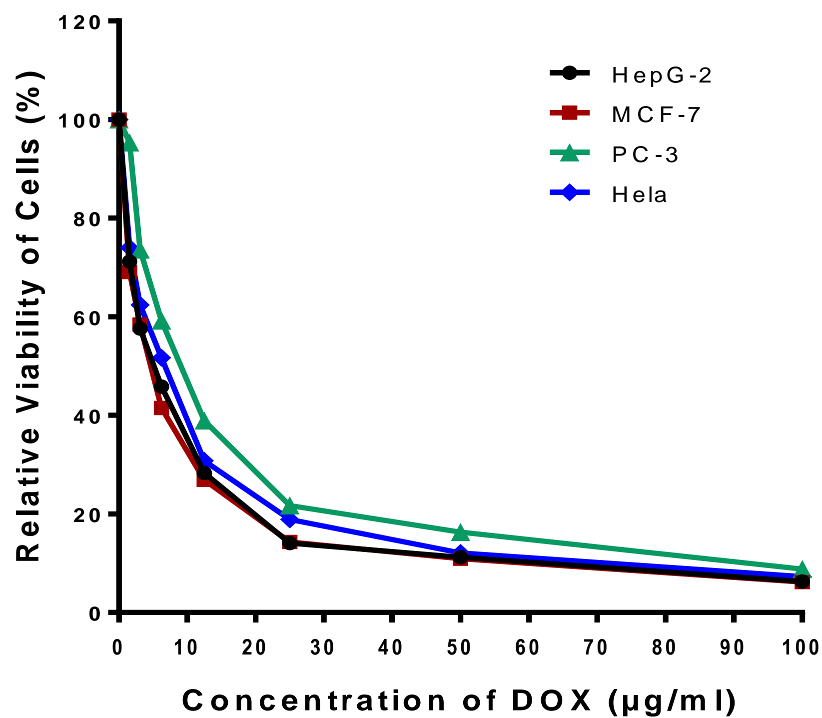

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Fig. 3. Relative viability cells (%) using different concentrations of H_2O extract of *A. heterophylla* against four human tumor cells.

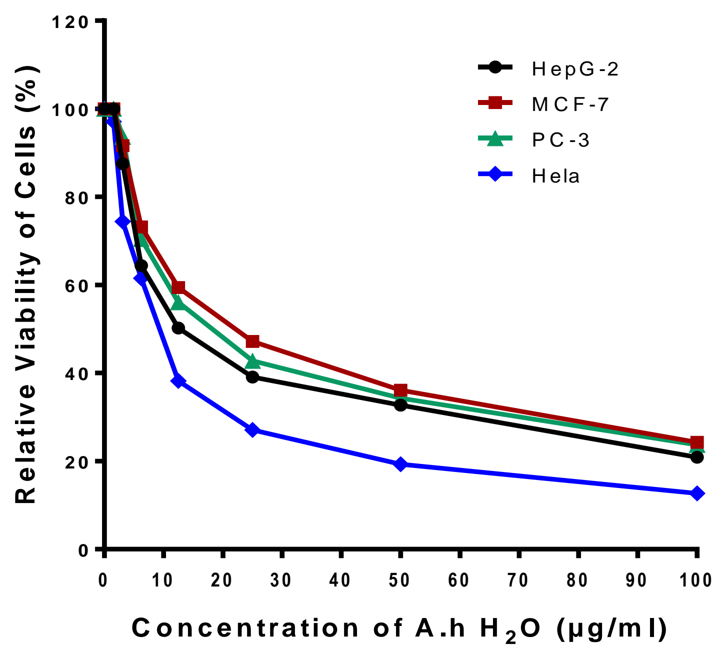

Fig. 4. Relative viability cells (%) using different concentrations of CH_2Cl_2 extract of *A. heterophylla* against four human tumor cells.

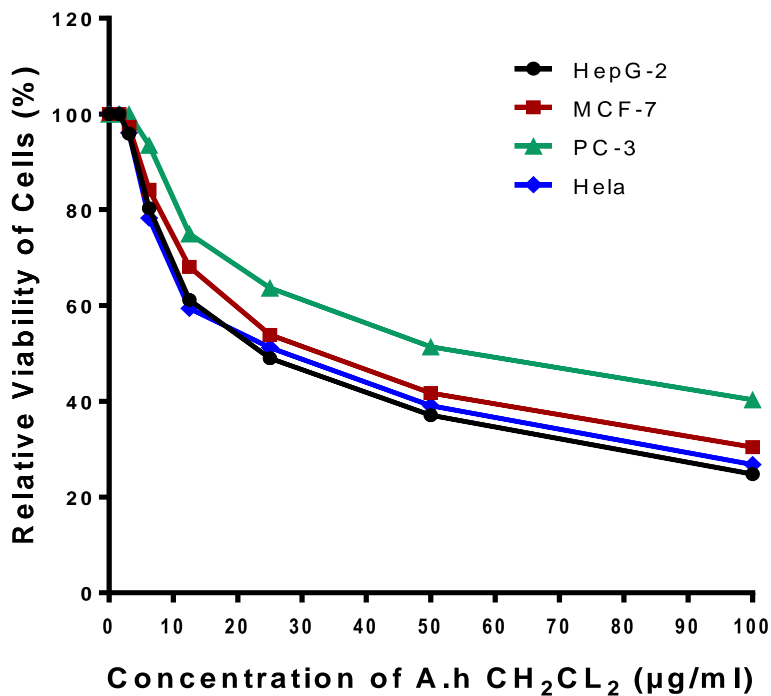

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Fig. 5. Relative viability cells (%) using different concentrations of BuOH extract of *A. heterophylla* against four human tumor cells.

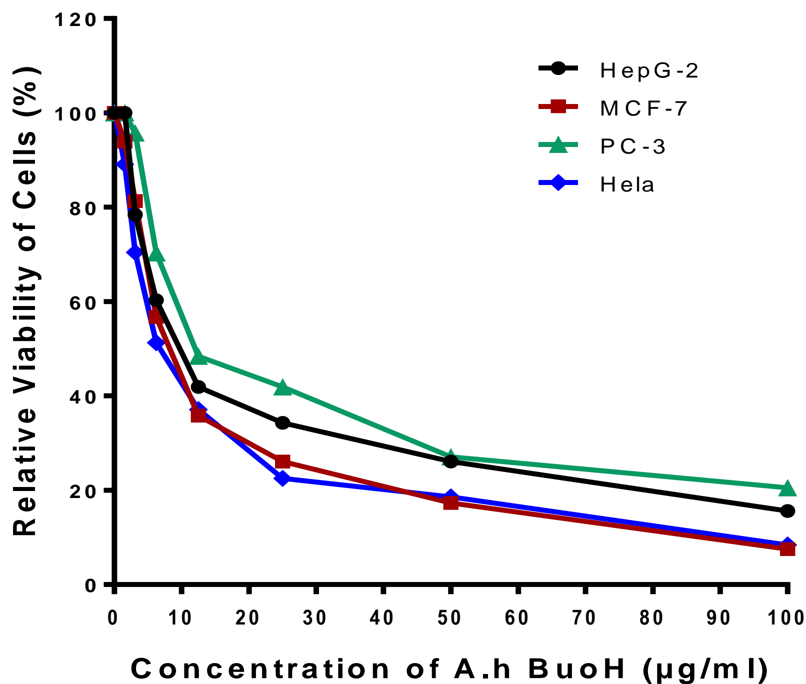

Fig. 6. Relative viability cells (%) using different concentrations of petroleum ether extract of *A. heterophylla* against four human tumor cells.

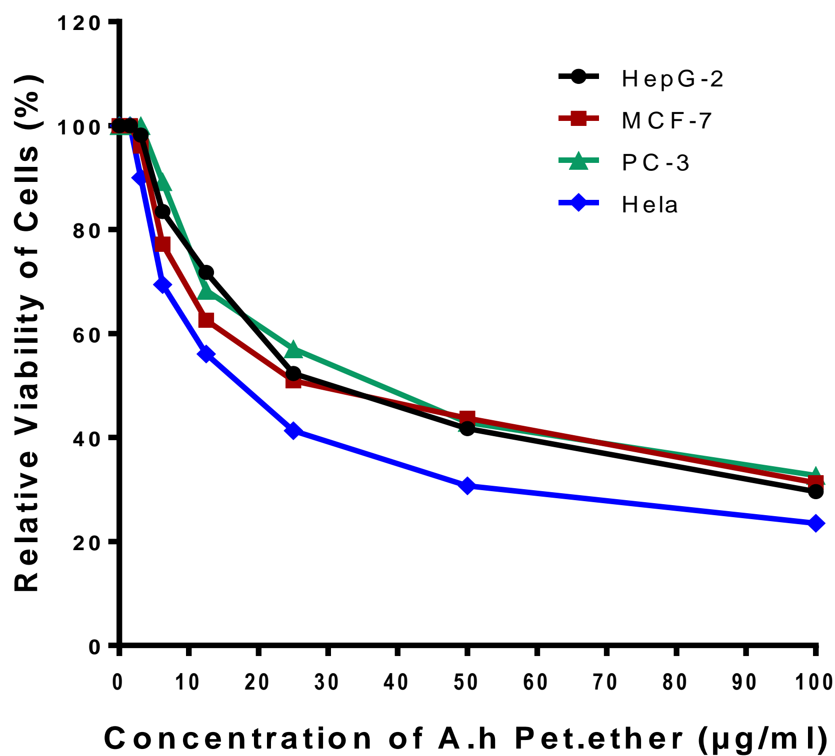

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Fig. 7. Relative viability cells (%) using different concentrations of ethyl acetate extract of *A. heterophylla* against four human tumor cells.

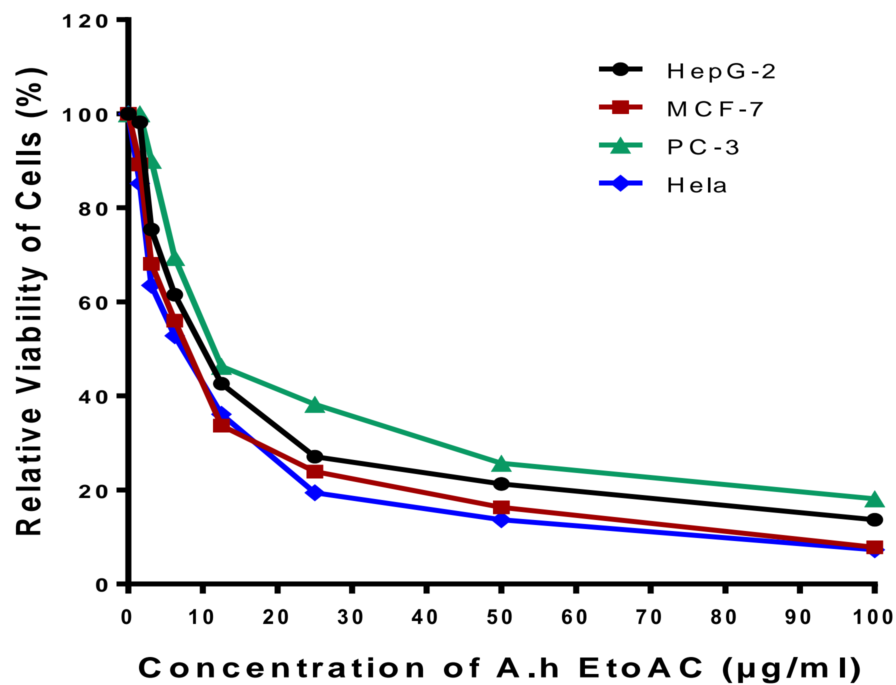
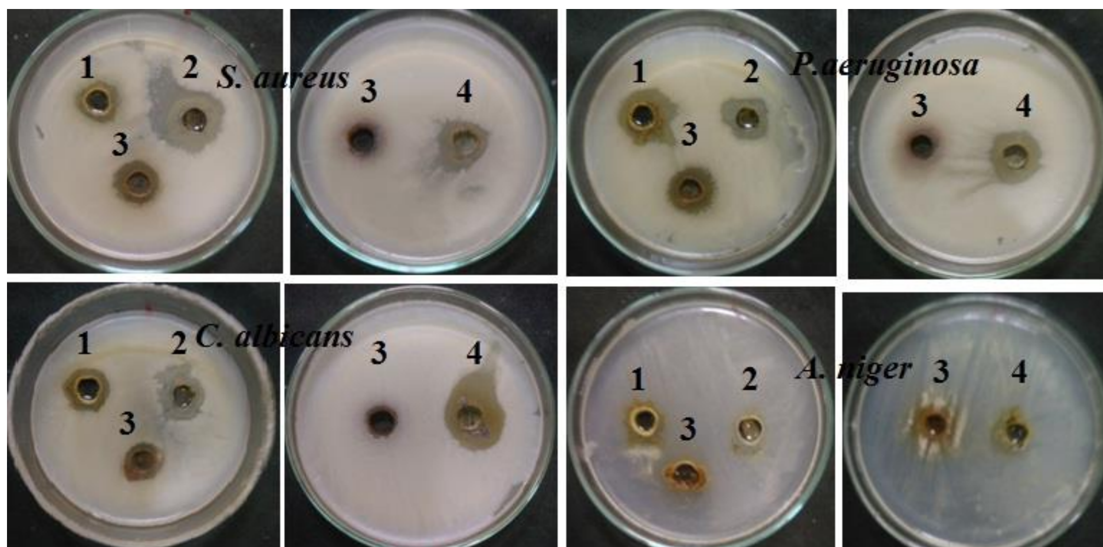


Fig. 8. Antimicrobial inhibition zones of the different solvent extracts of *A. heterophylla* leaves



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