



A New Insight into *Pleiogynium timorense* Leaves: A Focus on The Anticancer and Antimicrobial Potentials

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Abstract

The current study focused on the anticancer and antimicrobial potentials of the ethyl acetate extract of *Pleiogynium timorense* leaves and the characterization of its phytoconstituents. Ethyl acetate extract of *pleiogynium timorense* leaves showed a potent cytotoxic activity against ovarian cancer cell line (SKOV-3) with IC₅₀ of (7.13 µg/mL) and a moderate activity against liver cancer cell line (HEPG-2) with IC₅₀ =20.42 µg/mL, while it possessed a weak activity against Prostate cancer cell line (PC-3) (IC₅₀ >100 µg/mL). In addition, the extract exhibited reasonable antibacterial activity against *Staphylococcus aureus*, *Bacillus cereus*, and *Escherichia coli* with MIC values of (200, 500 and 500 mg/ml), respectively without the detection of any activity against *Salmonella typhimurium*, *Candida albicans* and *Aspergillus brasiliensis*. The phytochemical study revealed the identification of eleven polyphenolic compounds representing 80.8 % of the total area; the major compounds were chlorogenic acid (40.8 %), gallic acid (11.1%), catechin (8.2%) and taxifolin (7.6 %). Moreover, gallic acid, kaempferol and catechin were isolated and identified for the first time from the ethyl acetate extract. In addition, the plant extract is found to be rich in natural pigments (total carotenoids = 864.64 µg/100g). In conclusion, the current study revealed that ethyl acetate extract of *Pleiogynium timorense* leaves is rich with polyphenolic compounds, which play a vital role in the plant bioactivities.

Key words: *Pleiogynium timorense* leaves; cytotoxic; antimicrobial; phytoconstituents

Introduction

Cancer is the most important cause of death all over the world, cancer is known as a hyper proliferation of cell division responding appropriately to the signals that control normal cell behaviour, cancer cells grow and divide in an uncontrolled manner, spreading among normal cells and tissues then among all the body, this hyper proliferation of the cells are the result for causing different kinds of cancer [1]. Aiming to avoid the complications accompanied chemical drugs; many studies were carried out to discover drugs from plant origin, as plants possess relatively safer constituents. Nowadays, scientists use

active plant for treating some critical diseases like cancer by extracting the active cytotoxic materials from them [2].

The antimicrobial activity of different plant species and their chemical constituents against several microorganisms has been studied. The medicinal plants contain several phytochemicals such as tannins, triterpenes, alkaloids and flavonoids which showed a promising antimicrobial activity [3-5].

Pleiogynium timorense (DC.) Leenh. (gambozia), is belonging to family Anacardiaceae. The plant showed diversity of biological activities. The leaves of the plant showed hypoglycaemic, anti-

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inflammatory, antioxidant, and antimicrobial activities. Moreover, phenolic contents as flavonoids were isolated and identified from the leaves [6,7]. The fruits showed antioxidant activity [8]. The seeds and pericarp of gambozia showed hepato-renal protective, analgesic, antioxidant and anti-inflammatory activities, these activities due to the presence of different phenolic contents [9]. Lipoidal matter of the seeds was analyzed by the GC/MS analysis, 5, 24 (28)-cholestadien-24-methylen-3b-ol and α -amyrin were isolated and identified from gambozia seeds [10]. Furthermore, three new bioactive trihydroxy alkylcyclohexenones were isolated and identified from dichloromethane extract of the bark which showed activity against the A2780 human ovarian cancer cell line [11]. The volatile constituents possess a cytotoxic activity against breast (MCF7) and laryngeal (HEp2) human cancer cell lines and a lower effect on human hepatoma HepG2 cells [12]. A recent study revealed that the methanol extract of the seeds of *Pleiogynium timorense* showed antihyperglycaemic and antihyperlipidemic activities [13]. Recently, methanol extract of the bark showed cytotoxic, antihyperglycaemic, hepatorenal protective and antioxidant activity [14].

No evidence reports dealing with the cytotoxicity or antimicrobial activity of ethyl acetate extract of *Pleiogynium timorense* leaves. So the current study focused on the anticancer and antimicrobial potentials of the ethyl acetate extract of *Pleiogynium timorense* leaves and the characterization of its phytoconstituents.

Materials and Methods

Materials for phytochemical study

Plant material

The leaves of *Pleiogynium timorense* were collected from the Zoo, Giza, Egypt, and identified by Dr Mohammed El-Gebaly, Department of Botany, National Research Centre (NRC), Egypt. Voucher specimen was deposited in the Herbarium of NRC, possessing number 2001. The leaves were dried, powdered, and kept in dark well-closed containers.

Phytochemical analysis

Extraction and determination of percentage yield

The air dried powder of *Pleiogynium timorense* leaves (500g) was consecutively extracted with

solvents of increasing polarity; petroleum ether (60–80°C), diethyl ether, chloroform, ethyl acetate, and methanol (70%), respectively until complete extraction in a Soxhlet apparatus. Each extract was independently evaporated in a rotary evaporator. The percentage of extractives was calculated separately.

Phytochemical screening

Successive extracts were physically be examined and tested for their phytoconstituents by qualitative standard procedures as previously described by [15,16].

Total phenolic assay

The total phenolic content (TP) was determined by Folin–Ciocalteu colorimetric method using gallic acid as a standard [17]. It was expressed as milligrams of gallic acid equivalents (GAE)/g of the dry plant materials.

Total flavonoid assay

Total flavonoid content (TFC) was measured by using an aluminum chloride colorimetric assay [18]. A calibration curve was established using quercetin as a standard. TFC was expressed as mg quercetin equivalent (QE)/g of the dry plant materials.

Quantitative estimation of the natural pigments

Estimation of carotenoids and chlorophyll was determined colorimetrically according to [19].

HPLC analysis of flavonoids and phenolics of the ethyl acetate extract of *Pleiogynium timorense* leaves

HPLC analysis was carried out using an Agilent 1260 series. The separation was carried out using Kromasil C18 column (4.6 mm x 250 mm i.d., 5 μ m). The mobile phase consists of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate 1 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0–5 min (80% A); 5–8 min (60% A); 8–12 min (60% A); 12–15 min (85% A) and 15–16 min (82% A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 10 μ l for each of the sample solutions. The column temperature was maintained at 35 °C. Peaks were identified by congruent retention times and UV spectra in comparison with those of the standards [20].

General experimental conditions

Nuclear Magnetic Resonance spectrometers JEOL EX-500 spectrometer (Tokyo, Japan) with 500 MHz for ¹H-NMR. UV/VIS: Shimadzu UV-visible recording spectrophotometer model-UV 240 (NRC, Giza, Egypt). Sephadex LH-20 (Pharmacia Fine Chemicals). Thin layer chromatography (TLC) F₂₅₄ plates. Paper Chromatography (PC) Whatman No.1, (Whatman Led. Maid Stone, Kent, England), Solvent mixtures; S₁: (methanol: chloroform (30:70), S₂: (methanol: distilled water) (1:1), S₃: BAW (n-butanol: acetic acid: water) (4:1:5, v/v/v) upper phase), and S₄: (water: glacial acetic acid) (85:15, v/v).

Isolation and purification of the compounds

Ethyl acetate extract of *Pleiogynium timorense* leaves (30g) was subjected to preparative TLC using solvent mixture S₁ as developing system to afford three main bands. Each band was completely separated and extracted with S₂, then purified separately on Sephadex LH-20 column using methanol and different systems of methanol and distilled water (methanol: distilled water, 1:1, 2:1, v/v). Three compounds were isolated (one from each band), these compounds were compared with the available authentic flavonoids on TLC plates and PC to confirm the isolated compounds that have identical R_f values in different solvent systems with that of the authentic compounds.

Biological activity**In vitro cytotoxic activity****Cancer cell lines**

The cytotoxicity assay was carried out against human cancer cell lines such as; SKOV-3 (Ovarian cancer cell line), PC-3 (Prostate cancer cell line), HepG2 (liver cancer cell line). These cell lines were obtained from Nawah Scientific Inc., (Mokatam, Cairo, Egypt).

Cell culture

Cells were maintained in RPMI media and were supplemented with 100 mg/ml of streptomycin, 100 units/ml of penicillin and 10% of heat-inactivated fetal bovine serum in humidified, 5% (v/v) CO₂ atmosphere at 37 °C [21, 22].

Assay method for cytotoxic activity

Cell viability was assessed by SRB assay. Aliquots of 100 μL cell suspension (5 × 10³ cells) were in 96-well

plates and were incubated in complete media for 24 h. Cells were treated with another aliquot of 100 μL media containing drugs at various concentrations ranging from (0.01, 0.1, 1, 10, 100 μg/ml). After 72 h of drug exposure, cells were fixed by replacing media with 150 μL of 10% TCA and incubated at 4 °C for 1 h. The TCA solution was removed, and the cells were washed 5 times with distilled water. Aliquots of 70 μL SRB solution (0.4% w/v) were added and incubated in a dark place at room temperature for 10 min. Plates were washed 3 times with 1% acetic acid and allowed to air-dry overnight. Then, 150 μL of TRIS (10 mM) was added to dissolve protein-bound SRB stain; the absorbance was measured at 540 nm using a BMG LABTECH®- FLUO star Omega microplate reader (Ortenberg, Germany) [21, 22].

Antimicrobial activity**Microorganisms**

The microorganisms used in the *in vitro* antimicrobial study including bacterial and fungal strains were illustrated in **Table (1)**.

Table 1. Bacteria and fungi used for the antimicrobial assays

Bacterial strain	Fungal strain
<i>Staphylococcus aureus</i>	<i>Candida albicans</i>
<i>Bacillus cereus</i>	
<i>Escherichia coli</i>	<i>Aspergillus brasiliensis</i>
<i>Salmonella typhimurium</i>	

Methods of antimicrobial activity [23]**Preparation of the extract for biological assay**

A stock solution of 500 mg of the plant extract dissolved in 1 ml of distilled water was prepared.

Determination of the antibacterial activity

Well diffusion method was used to determine and screen the antimicrobial activity of all extracts. Petri dishes containing 20 mL nutrient agar culture medium were inoculated with 0.2 mL of a bacterial cell suspension matching a 0.5 McFarland standard solution. The suspension was uniformly spread using a sterile swab over the surface of the medium. Wells of 8 mm in diameter were made in the agar plates with a sterile glass Pasteur pipette and 0.1 mL of the stock solution was added into the wells in compare to amoxicillin. The plates were then incubated at 37 °C for 24 h. The antimicrobial activity was assayed by

measuring the diameter of the inhibition zone formed around the wells in mm. Each assay was performed at least in duplicate.

Determination of the antifungal activity

The *Candida albicans* was cultured on sabouraud dextrose agar and incubated at 30°C for 24 hours while the other fungus was cultured on potato dextrose agar slant for 5 days. Using a sterile loop, pure colonies of the *Candida* species were transferred into a tube containing sabouraud dextrose broth cultured for 24h at 30°C. For the other fungus spore suspension was prepared in 10ml distilled water. Using a hemocytometer, the suspension was adjusted to 2–5×10⁶ conidia/ml. Petri dishes containing 20mL sabouraud dextrose agar for *Candida albicans* or potato dextrose agar for other fungus were inoculated with 0.2ml of the strains. The suspension was uniformly spread using a sterile swab over the surface of the medium. Wells of 8 mm in diameter were made in the agar plates with a sterile glass Pasteur pipette and 0.1mL of the stock solution was added into the wells. The antifungal drug diflucan (10mg/ml) was used as a control. The plates were then incubated at 30°C for 48 h. The zone of inhibition was measured in millimeter.

Determination of the minimal inhibitory concentration

The Petri dishes were inoculated with the strains as described before. The tested extract was diluted at different concentrations and each concentration was placed in each well. The MIC was defined as the lowest concentration of the compound capable for preventing the microbial growth in the culture medium [23].

Result and discussion

Phytochemical study

Examination of successive extractives of *Pleiogynium timorense* leaves

The results of phytochemical screening of successive extractives of *Pleiogynium timorense* leaves were included in Table (2). It showed that sterols and/or triterpenes were present in all extracts. Carbohydrates and/or glycosides were detected mainly in methanol (70%) extract. Alkaloids were absent in all extractives of the plant organ. Saponins were detected in minute amounts in methanol (70 %) extract. Coumarins were detected in both chloroform and methanol (70%) extractives. While, tannins and flavonoids were detected mainly in ethyl acetate and methanol (70 %) extracts. In addition, ethyl acetate extract was rich in flavonoids. This result was similar to what were reported by [12, 14], which revealed the presence of carbohydrate, terpenoids, coumarins, saponins, flavonoids and tannins, in fruits and bark of *Pleiogynium timorense* while alkaloids were absent in both organs. These phytochemicals played a vital role in the bioactivity of medicinal plants [3-5].

The results compiled in table 3 revealed that the percentages of the extractives yielded to light petroleum, diethyl ether, chloroform, ethyl acetate and methanol (70%) were variable. It showed that the maximum extractive values were found in methanol and ethyl acetate (4.3 , 3.9%) respectively, as compared with those of the other extracts. In addition, from table 2, ethyl acetate extract was found to be rich with flavonoids. Therefore, ethyl acetate extract was chosen for further study.

Table 2. Results of phytochemical screening of successive extractives of *Pleiogynium timorense* leaves

Extract	Carbohydrate	Tannins	Flavonoids	Sterols/triterpenes	Saponins	Coumarins	Alkaloids
Pet. Ether (60-80 ° C)	-	-	-	++	-	-	-
Diethyl ether	-	-	-	+	-	-	-
Chloroform	-	-	-	+	-	+	-
Ethyl Acetate	-	+	++	+	-	-	-
Methanol (70 %)	++	+	+	+	±	+	-

- : The absence of the constituents

+ : The presence of the constituents

± : The presence of the constituents in minute amounts

++ Appreciably present

Table 3. Percentage of the extractive yield of *Pleiogynium timorense* leaves

Extract	Pet. Ether (60-80 ° C)	Diethyl ether	Chloroform	Ethyl Acetate	Methanol (70 %)
%W/W	0.9	0.3	0.7	3.9	4.3

Total phenolic and flavonoids contents

Total phenolic content was calculated as gallic acid equivalent and expressed as mg gallic acid/g extract. The results revealed that the total phenolic content (Gallic Acid Equivalent) of *Pleiogynium timorense* leaves = **134.56 mg / g** Extract (Standard Deviation = 6.8). On the other hand, the total flavonoid content was calculated as rutin equivalent and expressed as mg rutin/g extract. Total flavonoids (rutin equivalent) = **56.5 mg / g** Extract (Standard Deviation = 4). From this result, we can conclude that *Pleiogynium timorense* leaves is a rich source with polyphenolic compounds.

In a previous study, the total phenolic content of the fruits of *Pleiogynium timorense* was 15.6 mg gallic acid equivalent / g of the dry plant materials, while the total flavonoid was 12.3 mg quercetin equivalent (QE)/g of the dry plant materials [12].

Quantitative estimation of the natural pigments

The results compiled in table 4 showed the pigments profile in *Pleiogynium timorense* leaves. Total carotenoids represents the highest pigment content (**864.64 µg/100g**), followed by chlorophyll B (**4.278 µg/100g**); while chlorophyll A represents the lowest pigment content (**2.878 µg/100g**). These results revealed that *Pleiogynium timorense* leaves are a rich source of carotenoids, which were reported to have an effective role on human health [24].

Table 4: Natural pigments identified in *Pleiogynium timorense* leaves

Pigments content	Concentration (µg/100g)
Chlorophyll A	2.878
Chlorophyll B	4.278
Total carotenoids	864.64

HPLC Analysis of polyphenolic compounds of ethyl acetate extract of *Pleiogynium timorense* leaves

Polyphenolic compounds of ethyl acetate extract of *Pleiogynium timorense* leaves were evaluated by HPLC analysis, the result revealed the identification of eleven polyphenolic compounds representing 80.8 % of the total area, the major compounds were chlorogenic acid (40.8 %) , gallic acid (11.1), catechin (8.2 %) and taxifolin (7.6 %). This result was shown in table 5 and figure 1.

The previous research on *Pleiogynium timorense* revealed the identification of the constituents from both seeds and pericarp of *Pleiogynium timorense*

using High-Performance Liquid Chromatography with Electrospray Ionization Mass Spectrometry (HPLC–ESI–MS/MS) in negative ionization mode. Quercetin, catechin vanillic acid, coumaric acid, digalloylquinic acid, protocatechuic acid, gallic acid, galloylquinic acid, shikimic acid, malic acid, and quinic acid were detected in both pericarp and seed extracts of the plant [25]. A recent research on *Pleiogynium timorense* bark investigated the flavonoids and phenolic compounds by HPLC analysis, the result showed that sixteen phenolic compounds were identified representing 25.85 mg/g of the total content. Catechin was the major phenolic compounds (4.56 mg/g) followed by ρ -hydroxy benzoic acid (3.26 mg/g), while fourteen flavonoidal compounds were identified representing 36.97 mg/g of the total content, quercetin was the major flavonoid (5.31 mg/g) followed by naringenin (5.12 mg/g) [14].

Table 5. Identified polyphenolic compounds in ethyl acetate extract of *Pleiogynium timorense* leaves by HPLC analysis

Number	Compounds	Area %
1	Gallic acid	11.1
2	Chlorogenic acid	40.8
3	Catechin	8.2
4	Methyl gallate	2.7
5	Coumaric acid	1.8
6	Vanillin	1.3
7	Ferulic acid	2.7
8	Naringenin	1.4
9	Taxifolin	7.6
10	Cinnamic acid	1.8
11	Kaempferol	1.4
Total identified compounds		80.8%

Characterization of the isolated compounds

Compound (1): 12 mg, R_f values: = 0.68 in S_3 and 0.54 in S_4 , 1H -NMR spectral data are similar to those of (Abdel Raouf *et al.*,2020). It was obtained as white amorphous powder. Under short UV light, it gave violet color. Comparing with the authentic sample and with the published data [14], compound 1 was identified as Gallic acid.

Compound (2): (7 mg), R_f values: = 0.66 in S_3 and 0.9 in S_4 . It was obtained as yellow powder. Under UV and with $AlCl_3$, it gave dull yellow color. UV spectral data are similar to those published by [14,

26], and by comparing with the authentic sample, compound 2 was identified as kaempferol

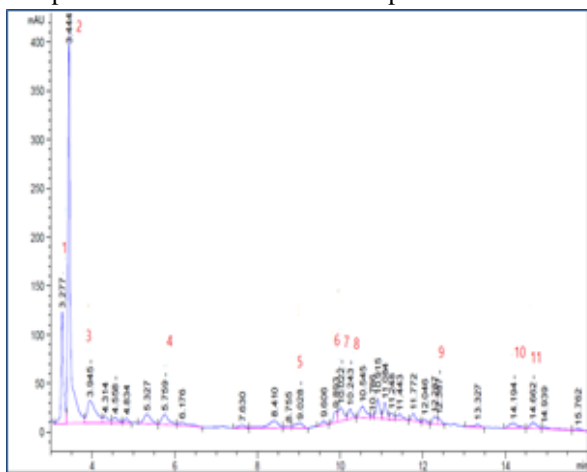


Figure 1. HPLC analysis of polyphenolic compounds in ethyl acetate extract of *Pleio gynium timorense* leaves

Compound (3): (8 mg), R_f value= 0.6 in S_1 , 0.78 in S_3 and 0.65 in S_4 . It was obtained as white crystals. It gave strong pink color with vanillin sulphuric acid reagent, $^1\text{H-NMR}$ spectral data are similar to those of [14, 27]. By comparing with the authentic sample (both gave the same color with vanillin sulphuric acid reagent and in different solvent systems, both have identical R_f values), compound 3 was identified as catechin.

Figure (2) showed the chemical structures of the isolated compounds.

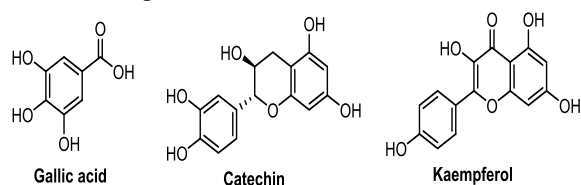


Figure 2. Chemical structures of the isolated compounds from ethyl acetate extract of *Pleio gynium timorense* leaves

Cytotoxicity activity

Treatments of cancers by herbal remedies are more useful than synthetic treatment. Herbal treatment is the best replacement for treating by avoiding various

Table 6. IC_{50} values ($\mu\text{g/ml}$) of ethyl acetate extract of *Pleio gynium timorense* leaves *in vitro* on different human cell lines

Tumor cell line	IC_{50} $\mu\text{g/ml}$	
	Ethyl acetate extract of <i>Pleio gynium timorense</i> leaves	Doxorubicin
HEPG-2	20.42	2.32
SKOV-3	7.13	0.97
PC-3	>100	5.37

physical side effects caused by the chemotherapy and radiation therapy like pain, nausea, vomiting, fatigue, anemia, lymph edema, fertility problems and ostomies caused by different cancer treatments [28]. Cytotoxic activity of ethyl acetate extract of *Pleio gynium timorense* leaves was investigated *in vitro* against different cancer cell lines in comparison with Doxorubicin as a reference anticancer agent. The results showed that the plant extract decreased the viability % of ovarian cancer cell line (SKOV-3) (figure3), prostate cell line (PC-3) (figure 4) and liver cancer cell line (HepG2) (figure 5) in dose dependent manner by comparing with that of Doxorubicin. These results revealed that ethyl acetate extract of *Pleio gynium timorense* leaves exhibited a reasonable cytotoxic activity in dose dependent manner.

The IC_{50} values ($\mu\text{g/ml}$) of ethyl acetate extract of *Pleio gynium timorense* leaves *in vitro* against the tested human cell lines were compiled in table 6 . The result showed that the plant extract exhibited a potent cytotoxic activity against the ovarian cancer cell line (SKOV-3) with IC_{50} of (7.13 $\mu\text{g/mL}$) and a moderate activity against the liver cancer cell line (HEPG-2) (IC_{50} =20.42 $\mu\text{g/mL}$) as compared with Doxorubicin, while it showed a weak activity against the prostate cancer cell line(PC-3) (IC_{50} >100 $\mu\text{g/mL}$) by comparing with that of Doxorubicin.

These results are in agreement with what were reported by various researchers who confirmed the effect of *Pleio gynium timorense* as a cytotoxic agent against different cancer cell lines [11, 12, 14]. A previous study showed the isolation of three new trihydroxy alkylcyclohexenones from dichloromethane extract of *Pleio gynium timorense* bark, these compounds showed activity against the A2780 human ovarian cancer cell line [11]. Another study revealed that the fruits of *Pleio gynium timorense* contained volatile constituents that showed a cytotoxic activity against laryngeal (HEp2) and breast (MCF7) human cancer cell lines and a lower effect on human hepatoma HepG2 cells [12]. A

recent study revealed the cytotoxic effect of the methanol extract of the bark against HepG2 cell line,

while gallic acid and catechin exhibited moderate effects [14].

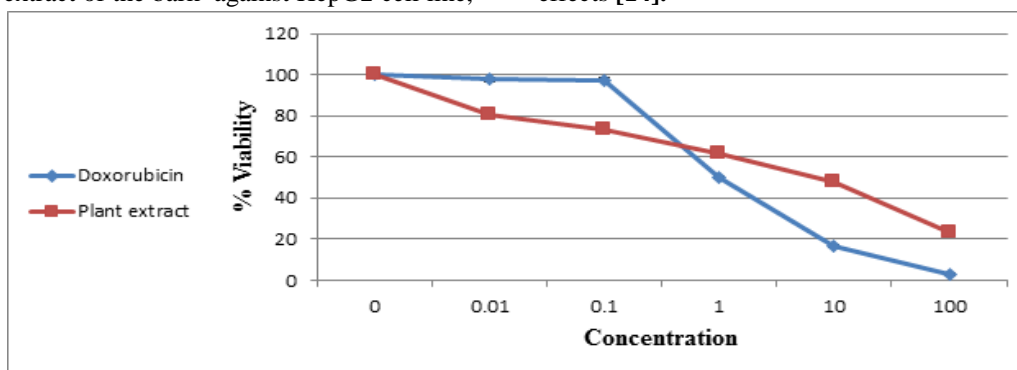


Figure 3. Cytotoxic activity of ethyl acetate extract of *Pleiogynium timorens* leaves against SKOV-3 human cell line *in vitro*

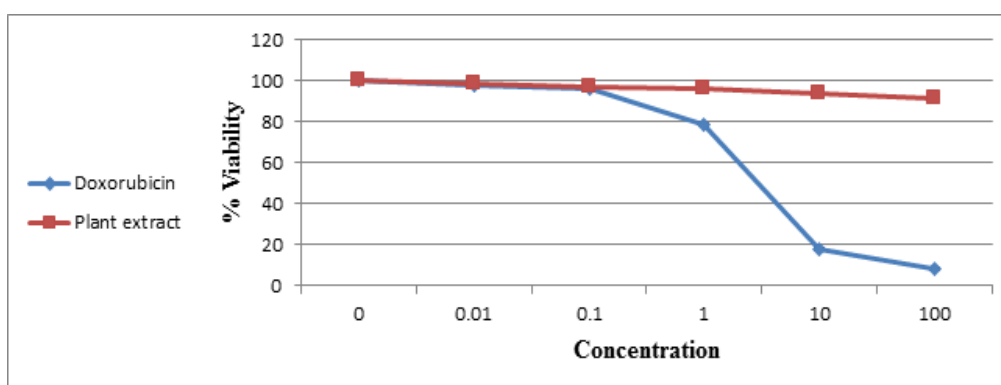


Figure 4. Cytotoxic activity of ethyl acetate extract of *Pleiogynium timorens* leaves against PC-3 human cell line *in vitro*

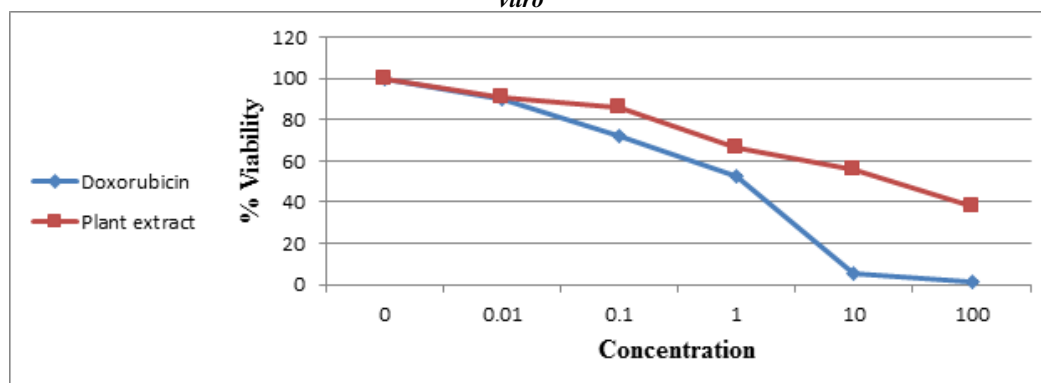


Figure 5. Cytotoxic activity of ethyl acetate extract of *Pleiogynium timorens* leaves against HEPG-2 human cell line *in vitro*

Antimicrobial activity

With the continuous use of antibiotics, microorganisms have become resistant. So, the development of alternative antimicrobial drugs is increasing interest. one approach is to screen local medicinal plants for possible antimicrobial properties [29]. The *in vitro* antimicrobial activity of the ethyl acetate extract of *Pleiogynium timorens* leaves was

screened for its *in vitro* antimicrobial activity against (*Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Salmonella typhimurium*, *Candida albicans* and *Aspergillus brasiliensis*). The results in table (7) showed that the extract exhibited reasonable antibacterial activity against (*Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli*) with an inhibition zone of 17, 12 and 12 mm respectively, as

compared with that of standard amoxicillin, while in case of *Salmonella typhimurium*, *Candida albicans* and *Aspergillus brasiliensis* the plant extract did not possess any antimicrobial activity.

The results also demonstrated that the ethyl acetate extract of *Pleiogynium timorense* leaves showed a potent antibacterial against (*Staphylococcus aureus*, *Bacillus cereus*, and *Escherichia coli*) with MIC values of (200, 500 and 500 mg/ml), respectively (table 8). These results were in agreement with what was reported by El-Fiki and Ahmed, it was found that aqueous and alcoholic extracts of air-dried

powdered leaves of *P. timorense* exhibited a significant antimicrobial activity against *Staphylococcus aureus* and *Bacillus subtilis* compared to the tested ampicillin [6]. This activity may be due to the phytoconstituents of *Pleiogynium timorense* leaves. Where, the phytochemicals such as tannins, triterpenes, alkaloids and flavonoids showed a promising antimicrobial activity [3-5]. In addition, the results revealed that the plant extract is a rich source in carotenoids that play a versatile biological role and are reported to have neuroprotective, antidiabetic, antibacterial, anti-inflammatory and anticancer effects [30].

Table 7. The antimicrobial activity of the ethyl acetate extract of *Pleiogynium timorense* leaves

Tested material	Diameter of the zone of inhibition (mm)			
	<i>Salmonella typhimurium</i> ATTCC 14028	<i>Escherichia coli</i> ATTCC 8739	<i>Bacillus cereus</i> ATTCC 14579	<i>Staphylococcus aureus</i> ATTCC 6538
EAEP	-	12	12	17
Amoxicillin (10mg/mL)	28	28	38	45

(-): no inhibition EAEP: Ethyl acetate extract of *Pleiogynium timorense* leaves

Table 8. The MIC values of ethyl acetate extract of *Pleiogynium timorense* leaves against bacterial strains

Bacterial Strain	<i>Salmonella typhimurium</i> ATTCC 14028	<i>Escherichia coli</i> ATTCC 8739	<i>Bacillus cereus</i> ATTCC 14579	<i>Staphylococcus aureus</i> ATTCC 6538
MIC (mg/ml)	Not detected	500	500	200

Conclusion

In the current research, it can be concluded that the ethyl acetate extract of *Pleiogynium timorense* leaves possess a potent cytotoxic activity against ovarian cancer cell line (SKOV-3) and a moderate activity against the liver cancer cell line (HEPG-2) as compared with doxorubicin, a reference anticancer agent. Moreover, the plant extract showed a promising antimicrobial activity against *Staphylococcus aureus*, *Bacillus cereus*, and *Escherichia coli*. The current study revealed that ethyl acetate extract of *Pleiogynium timorense* leaves is rich with polyphenolic compounds which play a vital role in the plant bioactivities. So, clinical researches are needed to confirm our study aiming to discover a safe drug from natural origin.

Conflicts of Interest

The authors have no conflict of interests to declare.

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