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Biological studies on chemical constituents of *Ruellia patula* and *Ruellia tuberosa*

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Abstract
The present study was carried out to evaluate various biological effects of the phytochemical constituents as well as extracts of *Ruellia patula* Jacq. and *Ruellia tuberosa* Linn. The study revealed that Rp-MeOH, Rp-Hexane, Rt-MeOH, Rt-Hexane and Rt-13 exhibited marked cytotoxic activity while, the compounds Rp-15, Rp-7, Rt-4 and Rt-12 exhibited moderate cytotoxic activity when compared with the positive control, doxorubicin. The compound Rt-13 showed a noticeable free radical scavenger activity, while Rp-EtOAc, Rp-7, Rp-15 and Rt-4 exhibited a moderate activity when compared to the reference compound, trolox. The compounds Rt-8, Rt-9 and Rp-14 showed approximately half the radical scavenger activity of the reference compound. The rest of the tested compounds did not exhibit any free radical scavenger activity. Rt-Hexane showed a weak antileishmanial activity while the remaining tested extracts and compounds showed no activity. All the tested extracts and compounds were found to exhibit no antibacterial activity.

Keywords: *Ruellia*, Acanthaceae, Cytotoxicity, DPPH, Antileishmanial.

1. Introduction
Family Acanthaceae, a large plant family known as Acanthus family, contains about 250 genera and about 2500 species. Members of this family found in hot countries, tropical and subtropical regions, Mediterranean region, Australia and USA [1-4]. The genus *Ruellia*, sometimes named *Dipteracanthus* [5], comprises about 250 species native to tropical and temperate North and South America [6]. *Ruellia patula* Jacq. [Syn. *Dipteracanthus patulus* (Jacq.) Nees.] and *Ruellia tuberosa* Linn. belong to family Acanthaceae. *R. patula* Jacq. is widely distributed in tropical Africa, Pakistan, south west India and Sri Lanka, while *R. tuberosa* Linn. is native of tropical America; introduced and naturalized in Africa, Pakistan, India and south west Asia [7]. In folk medicine, *R. patula* Jacq. was used in the treatment of gonorrhea, syphilis, eye sore, renal infection, cough, wounds, scalds, toothache, stomachache and kidney stones [8]. The kani tribes of Kilamalai, India used this plant as a remedy for the bite of special species of spider known as "Tiger spider" [9]. Furthermore, *R. tuberosa* has been used as diuretic, antidiabetic, antipyretic, analgesic, antihypertensive, thirst quenching and antidotal agent. The plant is also used to treat urinary problems and high cholesterol levels and it is used as anthelmintic and for estrus induction [10].
According to phytochemical studies of *R. patula* Jacq. and *R. tuberosa* Linn., it was proven that flavonoid glycosides together with lignans, phenolic glycosides, megastigmane glycosides, benzoxazinoid glucosides and sterols were the major constituents of the methanolic extract of the plants under investigation [11-12]. The various biological effects reported in literature, provoked us to carry out some other biological activities on the different plant extracts and the isolated compounds.

2. Materials and methods
2.1. Materials
2.1.1. Plant Material
The leaves of *R. patula* Jacq and *R. tuberosa* Linn were collected in July 2007 during the flowering stage of the plants from Orman Botanical Garden in Giza, Egypt. Voucher specimens were kept in a herbarium, Faculty of Pharmacy, Minia University, Minia, Egypt under title of (Minia-07-July-RP) and (Minia-07-July-RT), respectively [11-12].

2.1.2. Isolated compounds and extracts used

Compounds and extracts under investigation have been isolated in previous studies in our laboratory. They are listed in Table1 [11-12].

Table 1: A list of the isolated compounds and extracts from *R. patula* Jacq and *R. tuberosa* Linn.

Code	Name
Extracts and compounds isolated from *R. patula* Jacq.	
Rp-Hexane	Hexane extract of *R. patula*
Rp-EtOAc,	Ethyl acetate extract of *R. patula*
Rp-Butanol	Butanol extract of *R. patula*
Rp-Aq.	Aqueous extract of *R. patula*
Rp-MeOH,	Methanolic extract of *R. patula*
Rp-1	β-Sitosterol β-D- glucopyranoside
Rp-2	Vanilloside
Rp-3	Bioside (decaffeoyl verbascoside)
Rp-4	Byzantionoside B 6'-*O*-sulfate
Rp-5	Demethoxycentaureidin 7-*O*-β-D-galacturonopyranoside
Rp-6	Pectolinarigenin 7-*O*-α-L-rhamnopyranosyl-(1'"→4")-β-D-glucopyranoside
Rp-7	Acteoside
Rp-8	Syringin
Rp-9	Benzyl alcohol *O*-β-D-xylopyranosyl-(1"→2')-β-D-glucopyranoside
Rp-10	Cistanoside E
Rp-11	(6*S*,9*R*)-Roseoside
Rp-12	Phenethyl alcohol *O*-β-D-xylopyranosyl (1"→2')-β-D-glucopyranoside
Rp-13	(*Z*)-Hex-3-en-1-ol *O*-β-D-xylopyranosyl-(1"→2')-β-D-glucopyranoside
Rp-14	(-)-Lyoniresinol 3α-*O*-β-D-glucopyranoside
Rp-15	3,4,5-Trimethoxyphenol *O*-α-L-rhamnopyranosyl-(1"→6')-β-D-glucopyranoside
Rp-16	Isoacteoside
Rp-17	Pectolinarigenin 7-*O*-α-L-rhamnopyranosyl-(1'"→4")-β-D-glucopyranoside
Extracts and compounds isolated from *R. tuberosa* Linn.	
Rt-Hexane	Hexane extract of *R. tuberosa*
Rt-EtOAc,	Ethyl acetate extract of *R. tuberosa*
Rt-Butanol	Butanol extract of *R. tuberosa*
Rt-Aq.	Aqueous extract of *R. tuberosa*
Rt-MeOH,	Methanolic extract of *R. tuberosa*
Rt-1	β-Sitosterol β-D- glucopyranoside
Rt-2	3-hydroxy-1-(4-hydroxy-3-mehtoxyphenyl)-2-[4-(3-hydroxy-1-(E)-propenyl)-2-mehtoxyphenoxy] propyl-β-D-glucopyranoside
Rt-3	syringaresinol 4,4'-O-bis-β-D-glucopyranoside.
Rt-4	Acteoside
Rt-5	(2*R*)-2-*O*-β-D-glucopyranosyl-2H-1,4-benzoxazin-3(4H)-one
Rt-6	Syringin
Rt-7	(6S,9R)-Roseoside
Rt-8	(-)-Lyoniresinol 3α-O-β-D-glucopyranoside
Rt-9	Nepetin7-O-β-D-glucopyranoside
Rt-10	Cistanoside F
Rt-11	(2*R*)-2-*O*-β-D-glucopyranosyl-4-hydroxy-2H-1,4-benzoxazin-3(4H)-one
Rt-12	Demethoxycentaureidin 7-O-β-D-glucopyranoside
Rt-13	Pectolinarigenin 7-O- β-D-glucopyranoside

2.1.3. Microorganisms

The microorganisms used in this study, *Mucor racemosus*, methicillin-resistant *Staphylococcus aureus* and *Leishmania major*, were from Medical mycology research center, Chiba university, Japan and Institute of tropical medicine, Nagasaki university, Japan, respectively.

2.1.4. Cell line

Human lung cancer cell, A549 was obtained from the RIKEN Cell Bank, Japan.

2.1.5. Chemicals and media

Dulbecco's modified Eagle medium (DMEM) [Sigma-Aldrich Co., USA)], supplemented with 10% heat- inactivated fetal bovine serum (FBS) (Invitrogen, Co., USA). M-199 medium (Sigma-Aldrich Co., USA), supplemented with 10% heat-inactivated fetal bovine serum (FBS) and seeded with Leishmania promastigotes at 2x105 cells/100 µl of medium (L. major: MHOM/SU/73/5ASKH strain) From (National Bio-Resource Project, Japan). Kanamycin (Wako, Japan). Oxacillin (Wako, Japan). Amphotericin B (Sigma-Aldrich Co, USA). Doxorubicin (Wako, Japan). 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT Solution) (Nacalai, Japan). Müller-Hinton broth (Difco) (Sigma-Aldrich Co., USA). S)-(−)-6-hydroxy-2,5,7,8-tetramethylchlroman-2-carboxylic acid (Trolox) (Aldrich Chemical Co., Japan). 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Aldrich Chemical Co., Japan).

2.1.6. Equipment

VERSA max tunable UV-micro plate reader (Molecular Devices, USA). Asahi, 4020, CO2 incubator (Japan). Kubota, KR/702, centrifuge (Japan).

2.2. Methods

2.2.1. Human cancer cell growth inhibition assay

This assay was performed using human lung cancer cell line (A549) and the viability was estimated by the colorimetric MTT assay. Dulbecco's modified Eagle medium (DMEM) supplemented with fetal bovine serum (FBS) and 100 µg/ml of kanamycin and 5.6 µg/ml of amphotericin B was used as the cell culture medium. The test compounds were dissolved in DMSO and added to the each well of the 96-well micro-titration plates at 1% as final concentration. A549 cells (5×10^3 cells/well) were cultured in a 5% CO_2 incubator at 37 °C for 72 h. Then, MTT solution was added to each well and the plates incubated for a further 1.5 h. Then, the formazan precipitates were dissolved in DMSO and the optical density value for each well was measured at 540 nm with a microplate reader. Doxorubicin was used as a positive control. The cell growth inhibition was calculated using the following equation:

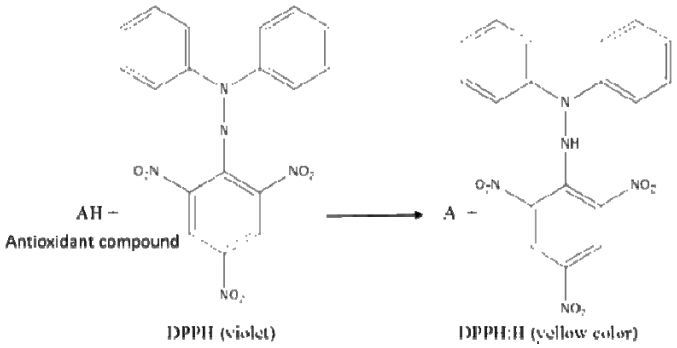
% Inhibition= [1 - (A sample - A blank) / (A control - A blank)] × 100

Where A control is the absorbance of the control reaction mixture (containing DMSO and all reagents except for the test compounds). IC_{50} was determined as the concentration of sample required to inhibit the formation of MTT formazan by 50% [13].

2.2.2. DPPH radical scavenging activity

The method is based on the reduction of methanolic 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution in the presence of a hydrogen donating antioxidant, due to the formation of the non-radical form DPPH-H by the reaction. The reduction in

DPPH radical was determined by the decrease in its absorbance at 515 nm induced by antioxidants, resulting in a color change from violet to yellow. This change in colour is a simple yet effective qualitative and quantitative parameter for the presence of an antioxidant. Hence, DPPH radical is used as a substrate to evaluate the antioxidative action of antioxidants [14].



The absorbance with various concentrations of the test extracts and compounds dissolved in MeOH (100 µl) in a 96-well micro-titration plate was measured at 515 nm at zero time as A blank. Then, 200 µM DPPH solution (100 µl) was add to each well, followed by incubation at room temperature for 30 min. The absorbance was measured again as A sample. The % inhibition was calculated using the following equation:

% inhibition= [1 - (A sample - A blank) / (A control - A blank)] ×100

Where A control is the absorbance of the control reaction mixture (containing DMSO and all reagents except for the test extracts and compounds). IC_{50} was determined as the concentration of sample required to inhibit the formation of the DPPH radical by 50% [15].

2.2.3. Anti-leishmania assay

The leishmanicidal activities of isolated compounds were performed using the colorimetric MTT assay. "Medium-199" medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 100 µg/ml of kanamycin was used as the cell culture medium. The test compounds were dissolved in DMSO and added to the each well of the 96-well micro-titration plates at 1% as final concentration. *Leishmania major* cells (2×105 cells /well) were cultured in a CO_2 incubator at 25 °C for 72 h. then, MTT solution was added to each well and the plates were incubated overnight at 25 °C. The absorbance was measured at 540 nm using a microplate reader. Amphotericin B was used as a positive control [16].

The inhibition % was calculated using the following equation:

% Inhibition= [1- (A sample – A blank) / (A control – A blank)] × 100

Where A control is the absorbance of the control reaction mixture (containing DMSO and all reagents except for the test compounds). IC_{50} was determined as the concentration of sample required to inhibit the formation of MTT formazan by 50%.

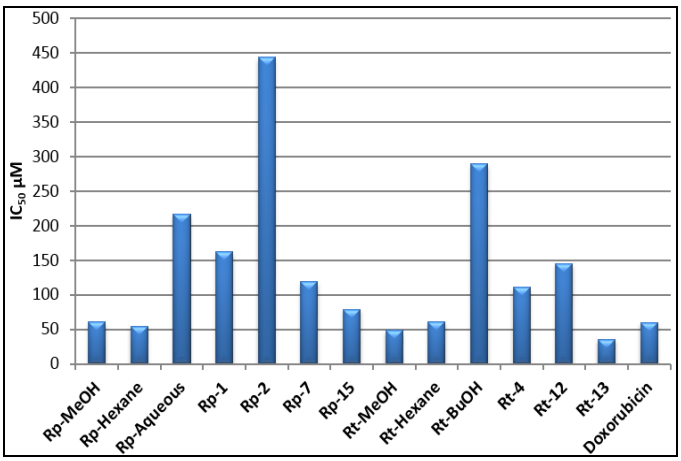
2.2.4. Antibacterial susceptibility assay

Susceptibility tests were performed using a broth microdilution assay according to the National Committee for Clinical Laboratory Standards (NCCLS) reference methods. Assays were performed using Müller-Hinton broth (Difco). The bacterial inocula were adjusted to yield a density of 5×10^5 colony forming units (CFU)/ml. Samples were diluted directly in 96-well micro-titration plates by serial 2-fold dilution using a multichannel pipette. Microtiter plates were incubated for 24 h at 37 °C and were read using microplate reader at 620 nm as well as by visual observation. The MIC_{50} was determined as a 50% decrease in the optical density. Amphotericin B and oxacillin were used as positive controls [13].

3. Results and Discussion

3.1. Determination of the cytotoxic activity

The extracts and compounds Rp-MeOH, Rp-Hexane, Rt-MeOH, Rt-Hexane and Rt-13 exhibited marked cytotoxic activity as compared with the positive control, doxorubicin, while the compounds Rp-15, Rp-7, Rt-4 and Rt-12 exhibited moderate cytotoxic activity as compared with the positive control. Furthermore, Rp-Aqueous, Rp-1, Rp-2 and Rt-BuOH showed very weak cytotoxic activity as compared with the positive control. On the other hand, the rest of the tested compounds showed no activity at all Fig.1.

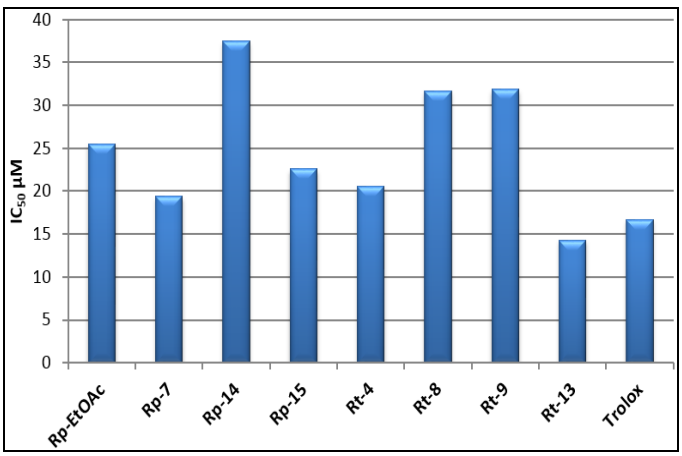


These results are mean of three replicates

Fig 1: The cytotoxic activity of the extracts and the isolated compounds on human lung cancer cell line (A549).

3.2. DPPH radical scavenging activity

Free radical-scavenging capacities of the test compounds were measured by DPPH assay. The compound Rt-13 showed noticeable inhibitory activity, while Rp-EtOAc, Rp-7, Rp-15, Rt-4 exhibited a moderate activity and compounds Rt-8, Rt-9 and Rp-14 showed approximately half the activity of the reference compound, trolox. The rest of the tested compounds did not exhibit DPPH radical scavenging activity Fig. 2.

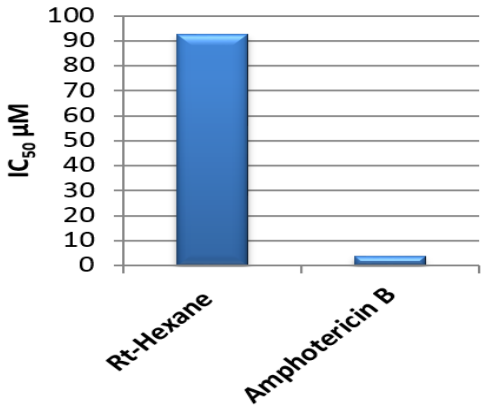


These results are mean of three replicates

Fig 2: DPPH radical scavenging activity of the tested extracts and isolated compounds.

3.3. Determination of the anti-leishmanial activity

It was found that only Rt-Hexane showed weak inhibitory activity, while the remaining tested extracts and compounds had no anti-leishmanial activity as compared with the positive control, amphotericin B Fig. 3.



These results are mean of three replicates

Fig 3: Anti-leishmanial activity of the extracts and the isolated compounds.

3.4. Antibacterial activity

All the tested extracts and compounds were found to have no antibacterial activity.

4. Conclusion

It was found that *R. patula* and *R. tuberosa* showed very close cytotoxic and DPPH radical scavenging activities. Only n-Hexane fraction of R. *tuberosa* that showed a very weak anti-leishmanial activity. On the other hand, both *R. patula* and *R. tuberosa* showed no antimicrobial activity.

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