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ANTICANCER, ANTIPLASMODIAL AND ANTITRYPANOSOMAL ACTIVITIES OF CRUDE EXTRACTS OF *PLATANUS ORIENTALIS*

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ABSTRACT

The present study evaluates the anticancer, antiplasmodial and antitrypanosomal potential of leaf extract of the buds and bark of *Platanus orientalis*. The buds and bark of the plant were extracted with dichloromethane. Concerning the cytotoxic activity, the two extracts of the plant have shown a good growth inhibition of cervical adenocarcinoma cell lines (HeLa cells), with an IC-50 of 10.1 µg/mL and 30.9 µg/mL, respectively. The activity was slightly lower for breast adenocarcinoma cell lines (MCF-7), with an IC-50 of 38.6 µg/mL and 31.6 µg/mL, respectively. The IC50 values were superior to 100 µg/mL for MDAMB-231 cells. The extracts showed also antiplasmodial (12.7 µg/mL for bark extracts and 35.3 µg/mL for buds extracts) and antitrypnosomal activities (20.0 µg/mL for bark extracts and 21.8 µg/mL for buds extracts).

KEYWORDS: *Platanus orientalis, Platanaceae,* buds, bark, anticancer, antiplasmodial, antitrypanosomal.

Platanus orientalis L. (Platanaceae) is a woody perennial tree, the buds of which are used in Georgiean folk medicine as urinary tract antiseptic [Bagrationi, D. 1993]. Particularly, *Platanus* species contain flavonoid aglycones, flavonoid glycosides, including acetylated and non-acetylated kaempferol mono glycosides, which are known to possess various biological activities such as astringent, antiseptic, anticancer, ophthalmic, and activity against urinary infections [Mitrokotsa D 1993, Imran Khan Payare L 2013, Dimas K, 2000]. Furthermore, secondary metabolites isolated from different plants showed a significant cytotoxic activity against murine fibro sarcoma, lung carcinoma, human melanoma and human leukemia cells [Mskhiladze L et al. 2008, Jabit ML et al. 2009, Zolfaghari B 2013].

According to the last World Malaria Report (WHO, 2013), there are almost 250 million malaria cases and about 600 000 people dying of malaria each year. Therefore, the discovery of new antimalarial drugs is urgently needed and natural products could play an important role in this new challenge [Jansen O et al. 2010, Jansen O et al. 2012].

Trypanosoma brucei is the parasite responsible for human African trypanosomiasis or sleeping sickness, an illness affecting 300,000–500,000 people, while up to 60 million people in 36 countries are at risk of contracting the disease [WHO]. In order to overcome this problem, traditionally used plants could also bring new active substances [Bero J et al. 2011]. The present study is the first report, on the *in vitro* anticancer, antiplasmodial and antitrypanosomal activity of *Platanus orientalis* growing in Georgia.

MATERIALS AND METHODS

Cell lines, chemicals and biochemicals

The human cancer cell lines, HeLa (ATCC), MDA-MB-231 (HTB-26, ATCC), MCF-7 (HTB-22 ATCC) and the normal lung fibroblasts WI-38 (ATTC) were grown in Dulbecco's Modified Eagle's Medium (DMEM) and Eagle's Minimum Essential Medium (EMEM) supplemented with 10% of fetal bovine serum, 1% L-glutamine, and non-essentials amino acid for EMEM. All the cells were grown at 37°C in a humidified atmosphere and 5% CO_2. Dimethyl sulfoxide (DMSO) was purchased from Sigma-Aldrich (Bornem, Belgium) as well as standard drugs: Doxorubicune, Artemisinin and Suramine. The cytotoxic activities of all samples was monitored on a UV-visible spectrophotometer (HP 8453, Waldbronn – Germany). The antiplasmodial and antitrypanosome activity was monitored by a multiwell scanner (Stat Fax 2100, Awareness Technology Inc).

Plant material

The buds and bark of *Platanus orientalis* were collected in Tbilisi, capital of Georgia (May 2013) and identified by Dr. Otar Shainidze (Department of Botany, Batumi Shota Rustaveli State University). A voucher specimen (# 02137) was deposited at the herbarium of the Department of Pharmaceutical Technology, Faculty of Pharmacy, Tbilisi State Medical University.

Extraction

A quantity of 50 grams of dried and powdered buds and bark of *Platanus orientalis* were extracted with dichloromethane at 70 °C during 2h. The extracts were filtered through filter paper, concentrated under vacuum, and then freeze-dried. The extracts were then stored in a dessicator prior to the assays.

Cancer cells viability analysis

Cell viability was determined using the cell proliferation reagent WST-1 according to the manufacturer's instructions (Roche, Basel, Switzerland). All analyzed cells were seeded to obtain 50% of confluence after 24h of incubation in 96-well plates, 6 × 103 cells per well and then treated for 24h with serial dilutions of the extract (5 - 50µg/mL). Cells were then incubated with WST-1 reagent for 4h. After this incubation period, the formazan dye formed was quantified by dual wavelength spectrophotometry at 450 nm. The measured absorbance directly correlates to the number of viable cells. The experiment was performed in triplicate for each sample.

Antiplasmodial assays

The culture of *Plasmodium falciparum* strains and the antiplasmodial assay was carried out as previously described by Frederich et al., 2001. The 3D7 plasmodial strain was obtained from ATCC-MR4 reagents (USA). [Frederich et al. 2001; Jonville et al 2008]. Artemisinin (98%, Sigma–Aldrich) was used as reference standard (IC50 0.015 ± 0.008 µg/mL). The results were expressed as the mean IC50 (the concentration of a drug that reduced the level of parasitaemia to 50%). All tests we performed in triplicate.

Antitrypanosomal assays

Trypanosoma brucei brucei (strain 427) bloodstream forms were cultivated *in vitro* in a modified Iscove's medium containing 10% heat-inactivated fetal calf serum (HIFCS) and bloodstream form supporting factors: 0.05mM bathocuproine sulfonate, 1.5mM l-cysteine,

1mM hypoxanthine, 0.2mM 2-mercaptoethanol, 1mM sodium pyruvate,0.16mM thymidine known as HMI 9 [Hirumi and Hirumi, 1994]. Suramin (99%, Sigma–Aldrich) was used as standard (IC50 0.059 ± 0.004 µg/mL). All tests we performed in triplicate.

RESULTS AND DISCUSSION

The present study evaluates the anticancer, antiplasmodial and antitrypanosomal potential of extracts of the buds and bark of *Platanus orientalis*. The buds and bark of the plant were extracted with dichloromethane and after evaporation of the solvent, the residue was lyophilized.

Concerning the cytotoxic activity, all extracts of the plant have shown a good inhibition of the growth of breast adenocarcinoma cell lines, with an IC-50 of 38.6 µg/mL and 31.6 µg/mL for MCF-7 cells; for MDA MB-231 the IC-50 values were higher (>100µg/mL). For the cervical adenocarcinoma cell lines (HeLa), the IC-50 were of 10.1 µg/mL and 30.9 µg/mL while doxorubicin, used as standard drug, showed an IC-50 of 2.0 µg/mL. In vitro anticancer activities are summarized in Table 1.

Regarding then the antiplasmodial activity, the bark extract showed a strong activity, with an IC-50 of 12.7µg/mL, which could be considered as highly active for a crude extract, while buds extract showed a moderate activity with an IC50 value of 35.3 µg/mL. The reference compound, artemisinin, showed a pronounced activity (IC-50 = 0.004 µg/mL).

Finally, the antitrypanosomal activity of the extracts was also quite interesting with IC50 of 20.0 µg/mL and 21.8 µg/mL, while suramin, used as standard drug, showed a pronounced activity (IC-50 = 0,059 µg/mL). *In vitro* antiplasmodial and antitrypanosomal activity are summarized in Table 2.

This is the first report of the antiplasmodial, antitrypanosomal and anticancer activities (on solid tumor cells) of *Platanus orientalis*, which grows in Georgia. The buds were already shown to be active on human Leukemic cells [Dimas et al., 2010]. According to the results obtained, the properties of *Platanus orientalis* need to be further investigated by isolation and identification of pure bioactive compounds by bio-guided fractionation. Some flavonoid and betulinic acid derivatives have already been isolated from this species and could contribute to the activities [Dimas et al., 2010; Imran Khan Payare et al., 2013].

Legends for Table

Table 1. *In vitro* anticancer activity of the extracts of *Platanus orientalis* and Doxorubicin.

	IC-50 WI-38 ± S.D. µg/ml	IC-50 MCF-7 ± S.D. µg/ml	IC-50 MDAMB-231 ± S.D. µg/ml	IC-50 HeLa ± S.D. µg/ml
Buds extract of *P.orientalis*	26.3 ± 3.7	31.6 ± 8.7	> 100	30.9 ± 2.9
Bark extract of *P.orientalis*	18.2 ± 3.7	38.6 ± 1.8	> 100	10.1 ± 1.8
Doxorubicin	n.d.	2.8 ± 0.4	4.2 ± 0.7	2.0 ± 0.5

* n.d.= not determined

Table 2. *In vitro* antiplasmodial and antitrypanosomal activity of the extract of *Platanus orientalis* and Standard drugs.

	Antiplasmodial IC-50 3D7 ± S.D.µg/ml	Antitrypanosomal IC-50 TREU667 ± S.D.µg/ml
Buds extract of *P.Orientalis*	35.3 ± 3.8	21.8 ± 1.6
Bark extract of *P.Orientalis*	12.7 ± 2.1	20.0 ± 0.9
Artemisinin	0.0042± 0.002	n.d.
Suramine	n.d.	0.059 ± 0.004

* n.d.= not determined

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Conflict of Interest

All authors of this paper confirm that they have no conflicts of interest.

REFERENCES

1. Bagrationi, D. Iadigar Daudi; Edition of Tbilisi University: Tbilisi, Republic of Georgia, 1993.
2. Mitrokotsa D, Mitaku S, Demetzos C, Harvala C, Mentis A, Perez S, Kokkinopoulos D. Bioactive compounds from the buds of Platanus orientalis and isolation of a new kaempferol glycoside. Planta Med, 1993; 59(6): 517-520.
3. Imran Khan Payare L, Sangwan Alamgir A, DarRather A, RafiqMufti R, Farrukh Jagdish K, Dhar Sheikh A, Tasduq Surrinder K. A validated high-performance thin-layer

chromatography method for the identification and simultaneous quantification of six markers from *Platanus orientalis* and their cytotoxic profiles against cancer cell lines. J. Sep. Sci, 2013; 36: 2602–2610.

4. Dimas K, Demetzos C, Mitaku S, Marselos M, Tzavaras T, Kokkinopoulos D. Cytotoxic activity of kaempferol glycosides againt human leukaemic cell lines *in vitro*. Pharmacological Research, 2000; 41(1): 85-88.
5. Mskhiladze L, Legault J, Lavoie S, Mshvildadze V, Kuchukhidze J, Elias R, Pichette A. Cytotoxic steroidal saponins from the flowers of *Allium leucanthum*. J. Molecules, 2008; 13: 2925-2934.
6. Jabit ML, Wahyuni FS, Khalid R, Israf DA, Shaari K, Lajis NH, Stanslas J. Cytotoxic and nitric oxide inhibitory activities of methanol extracts of *Garcinia* species. Pharmaceutical Biologi, 2009; 47(11): 1019-1026.
7. Zolfaghari B, Sadegui M, Troiano R, Lanzotti V. Vavilosides A1/A2-B1/B2, New furostane glycosides from the bulbs of *Allium vavilovii* with cytotoxic activity. Bioorganic & Medicinal Chemistry, 2013; 21: 1905-1910
8. WHO, 2013, World Malaria Report 2013, WHO, Geneva.
9. Jansen O, Angenot L, Tits M, Nicolas JP, DeMol P, Nikiema JB, Frédérich M. Evaluation of 13 selected medicinal plants from Burkina Faso for their antiplasmodial properties. Journal of Ethnopharmacology, 2010; 130: 143-150.
10. Jansen O, Tits M, Angenot L, Nicolas JP, DeMol P, Nikiema JB, Frédérich M. Anti-plasmodial activity of *Dicoma tomentosa* (Asteraceae) and identification of urospermalA-15-O-acetate as the main active compound. Malaria Journal, 2012; (11): 289.
11. Bero J, Hannaert V, Chataigne G, Hérent MF, Quetin-Leclercq J. In vitro antitrypanosomal and antileishmanial activity of plants used in Benin in traditional medicine and bio-guided fractionation of the most active extract. Journal of Ethnopharmacology, 2011; 137: 998– 1002.
12. Frederich M, De Pauw MC, Prosperi C, Tits M, Brandt V, Penelle J, Hayette MP, DeMol P, Angenot L. Strychnogucines A and B, Two new antiplasmodial bisindole alkaloids from *Strychnos icaja*. J. Nat. Prod, 2001; 64: 12-16.
13. Jonville C, Kodja H, Humeau L, Fournel J, DeMol P, Cao M, Angenot L, Frederich M. Screening of medicinal plants from Reunion Island for antimalarial and cytotoxic activity. J Ethnopharmacol, 2008; 120: 382-386.
14. Hirumi, H., Hirumi, K. Axenic culture of African trypanosome bloodstream forms. Parasitology Today, 1994; 10: 80-84.