SHORT REPORT

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Chemical and Biological Investigation of *Ochrosia elliptica* Labill. Cultivated in Egypt

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Abstract: The phytochemical investigation of *Ochrosia eliptica* leaves resulted in isolation and identification of eight compounds; lupeol, lupeol acetate, uvaol, ursolic acid, *β*-sitosterol glucoside, rutin, 8-methoxy and 9-methoxy ellipticine. The ethanolic extract and fractions were studied for their cytotoxic, antioxidant and anti-inflammatory activities. The cytotoxic activity was performed on human mammary adenocarcinoma (MCF7), its multidrug-resistant counterpart (VCREMS), estrogen receptor negative human metastatic breast adenocarcinoma cells (MDA-MB-231) and the non-cancerous, immortalized by telomerase, human breast epithelial cell line (hTERT-HME1). Additionally, the inhibitory potential on Cdc25s proteins was determined. The results showed that the dichloromethane (DCM) fraction and the major alkaloid; 9-methoxyellipticine exhibited high inhibitory activity against all tested cell lines particularly MCF7 and VCREMS cell lines, whereas the DCM fraction showed a significant inhibitory action on Cdc25 A isoform. In contrast, the *n*-butanol fraction and 9-methoxyellipticine displayed the highest antioxidant potential. The DCM fraction showed significant anti-inflammatory activity compared to indomethacin. This work comprises the first comprehensive work to be conducted on *O. elliptica* leaves showing its potential in multiple biological activities.

Keywords: *Ochrosia elliptica*; 9-methoxyellipticine; cytotoxicity; Cdc25s; anti-inflammatory.

1. Plant Source

Ochrosia elliptica Labill. is a small tropical evergreen tree native to Oceania [1] and cultivated in Egypt for ornamental purposes. The leaves were collected from El-Orman Botanical Garden, Giza, Egypt. The plant was authenticated by Dr. Reem Samir Hamdy, Associate Professor of Taxonomy and Flora, Department of Botany, Faculty of Science, Cairo University and a voucher specimen (no. 28.12.2012) was deposited at the herbarium of the department of Pharmacognosy, Faculty of Pharmacy, Cairo University.

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2. Previous Studies

O. elliptica is well known for ellipticine series of indole alkaloids isolated from the leaves and bark [1, 2], in addition to lignans, coumarins and phenolic acids [3]. Previous biological activities were concerned mainly with the cytotoxic activities of ellipticine [4]. Ellipticine and its derivatives primarily act via combined mechanisms of cell cycle arrest and induction of apoptotic pathways [5] against several cancer cell lines [6-9]. Herein, we thus report the cytotoxic activity of the ethanolic extract, as well as, the dichloromethane, *n*-butanol and alkaloidal fractions of *O. elliptica* leaves and the major isolated alkaloid against MCF-7, MDA-MB-231, VCREMS, hTERT-HME1 cell lines focusing on their cdc25s phosphatase inhibitory activity, along with determination of their antioxidant and *in-vivo* anti-inflammatory activities; in an effort to provide further insight into this plant's profile.

3. Present Study

The ethanolic (95%) extract of the leaves was fractionated into petroleum ether, dichloromethane (DCM) and *n*-butanol (*n*-BuOH). Based on a preliminary cytotoxic and antioxidant activity testing, the most active DCM and *n*-BuOH fractions were selected for further isolation procedures. The DCM fraction afforded compounds **1** - **5**. Compounds **6** and **7** were isolated from the *n*-BuOH fraction. Compounds **8** and further amounts of compound **7** were isolated from the crude alkaloidal fraction.

Identification of the isolated compounds: The structures of compounds **(1-8)** were determined via MS, ^{1}H and ^{13}C NMR spectral analysis and identified as lupeol acetate (**1**), lupeol (**2**), uvaol (urs-12-ene-3, 28-diol) (**3**), ursolic acid (**4**), *β*-sitosterol glucoside (**5**), rutin (**6**), 8-methoxyellipticine (**7**) and 9-methoxyellipticine (**8**) (See supporting information). Interestingly, compounds **1-3, 5-6** were previously reported in different plants belonging to family Apocynaceae [10-12], nevertheless this is the first report for their isolation from *O. elliptica*. 8-Methoxyellipticine (**7**) was previously obtained through microbial transformation [13] and chemical synthesis from ellipticine [14], as well as, suggested to be isolated from *O. sandwicensis* [15]. Herein, we report the full assignement of 8-methoxyellipticine for the first time. 9-Methoxyellipticine was previously isolated from the barks of *O. elliptica* [2]. Structures of isolated compounds are depicted in Figure **1**.

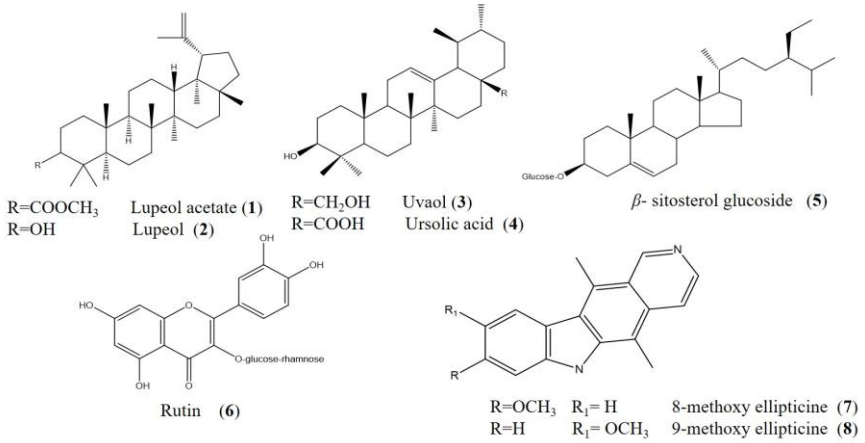

Figure 1. Structures of the isolated compounds from *O. elliptica* leaves

Cytotoxicity effects on cells: The results of the cytotoxic activity of the ethanolic, DCM, *n*-BuOH and the alkaloidal fractions, as well as, 9-methoxy ellipticine are summarized in Table 1. The tested samples showed a dose-dependent cytotoxic activity. The DCM fraction displayed the highest cytotoxic activity against all tested cell lines at a dose of 200 µg/mL with IC_{50} values of 0.31 ± 0.11, 25.95 ± 2.11 and 48.85 ± 5.49 µg/mL for MCF7, VCREMS and MDA-MB-231 cell lines, respectively followed by the alkaloidal and *n*-BuOH fractions exhibiting moderate to weak activity. Whereas, the major isolated alkaloid; 9-methoxyellipticine showed outstanding cytotoxic activity against all tested tumour cell lines

with IC_{50} values of 0.39, 1.9 and 0.39 µmol for MCF7, VCREMS and MDA-MB-231, respectively. The non-cancerous cell line hTERT-HME1 was very sensitive to all the tested samples with 76.7 to 95% loss of cell viability. The results obtained for the DCM fraction are in agreement with the previous reports for the capability of pentacyclic triterpenes of the lupane and ursane types to act as multitarget agents that can assist in cancer treatment from different approaches [16-17], in addition, ellipticine was reported to inhibit the growth of MCF7 and MDA-MB-231 cell lines [7, 18]. It is interesting to note that, although 9-methoxyellipticine was the major compound in the alkaloidal fraction, nevertheless, it showed potent cytotoxic activity in contrast to the moderate activity observed for its parent fraction.

Table 1. *In-vitro* cytotoxic activity (IC_{50}) of the extracts and alkaloidal fractions of *O. elliptica*

Test sample	IC_{50} (Mean ± S.D.) Cell lines			
	MCF7	**VCREMS**	**MDA-MB-231**	**hTERT-HME1**
Ethanol extract	ND	ND	ND	ND
DCM fraction	**0.31 ± 0.11**	25.95 ± 2.11	48.85 ± 5.49	48.38 ± 1.7
n-BuOH fraction	ND	ND	ND	ND
Alkaloidal fraction	31.16 ± 12.8	63.34 ± 5.68	40.22 ± 8.35	53.45 ± 27.9
9-methoxyellipticine (**8**)	0.11 ± 0.02 (0.3985)	0.34 ± 0.01 (1.90)	0.11 ± 0.01 (0.3985)	0.11 ± 0.03(0.3985)
SV37	29.63 ± 0.7 (11.2)	ND	25.132 ± 1.0 (9.5)	47.883 ± 1.3 (18.1)

ND: not determined; IC_{50}: µg/mL (µM).
SV37: 2{(7-Methoxy-2-oxo-2H-Chromen-yl) Methyl sulfanyl}Naphtoquinone

In vitro inhibition of phosphatase assay: The ethanolic, DCM and *n*-BuOH fractions of *O. elliptica* were evaluated for their ability to inhibit the activities of human Cdc25 A, B and C isoforms. The samples were tested at a dose of 4 mg/mL and the results are expressed as percentage of residual activity of Cdc25 phosphatases (Table 2). The obtained results indicated that the highest inhibitory activity was established for the DCM fraction followed by the ethanolic extract and finally the *n*-BuOH fraction compared to naphthoquinone as standard drug. The most potent inhibitory effect was observed against Cdc25A isoform, whereas Cdc25B was the least sensitive protein. Cdc25 phosphatases are considered interesting candidates within the context of research for new cancer therapy targets. Their inhibition allows slowing down of tumour growth and eventually improving cancer treatments. Numerous studies have associated cancer with over-expression of Cdc25 isoforms with this over-expression often being related to unfavourable prognosis [19]. Cdc25A and B have been described as oncogenes; since these proteins were upregulated in numerous tumours, including breast [20], hepatocellular [21] and other tumours. Considering the results described herein, the DCM fraction and its isolated compounds could be regarded as potential Cdc25 phosphatase inhibitors, in particular for Cdc25 A, which is in agreement with previous reports for lupeol [22], as well as ellipticine, which showed the ability to arrest MDA-MB-231 cells with concomitant reduction in the expression of cyclin B, Cdc25 and Cdc2 levels [7].

Table 2. Inhibitory activity of the extracts of *O. elliptica* leaves against Cdc25s phosphatases

Sample (4 mg/mL)	**% Residual activity (Mean ± S.D.)**		
	Cdc25 A	**Cdc25 B**	**Cdc25 C**
Ethanol extract	5.23 ± 0.74	41.78 ± 4.69	23.59 ± 5.05
DCM fraction	**4.80 ± 0.94**	32.37 ± 7.17	17.17 ± 7.25
n-BuOH fraction	11.54 ± 1.52	55.15 ± 13.88	32.28 ± 7.59
Naphthoquinone	0.01 ± 2.16	0.015 ± 1.67	0.03 ± 2.52

Values are means of three independent experiments

In-vitro antioxidant activities: The antioxidant activity of the samples was evaluated using DPPH and FRAP assays. Trolox was used as a reference (Table 3). The highest DPPH radical scavenging potential was observed for the *n*-BuOH fraction demonstrating an activity corresponding to 121.1 ± 4.9 µmol Trolox equivalent/g of the extract. While the results of the FRAP assay showed that 9-methoxyellipticine exhibited a remarkable 7.1-fold increase in ferric reducing power, followed by the *n*-BuOH and DCM fractions showing 2- and 1.7-fold increases in reduction efficiency compared to

Trolox, respectively. Antioxidant agents usually function as inhibitors at both initiation and promotion/propagation/transformation stages of carcinogenesis, consequently protecting cells against oxidative damage [23]. The significant radical scavenging activity observed for the *n*-BuOH and DCM fractions can be attributed to their chemical constituents, where antioxidant activities have been previously reported for rutin [24], uvaol [17], lupeol and ursolic acid [16]. To the best of our knowledge, this is the first report of the antioxidant activity of *O. elliptica* leaves and 9-methoxyellipticine.

Table 3. Anti-oxidant activity by DPPH and FRAP assays of the extracts and alkaloidal fractions of *O. elliptica* leaves

Sample	µmol Trolox eq./g extract	Anti-oxidant ratio*
Ethanol extract	25.2 ± 9.3	1.8 ± 0.00
DCM fraction	47.5 ± 9	**3.7 ± 0.28**
n-BuOH fraction	**121.1 ± 4.9**	**4.4 ± 0.2**
Alkaloidal fraction	26.4 ± 2.2	0.4 ± 0.05
9-methoxyellipticine (**8**)	0.42 ± 0.12	860 ± 0**
Trolox	-	2.1 ± 0.01

*Quantity of Fe^{2+} (nmol) reduced by 1 µg extract **Quantity of Fe^{2+} (nmol) reduced by 1 mg extract, Mean± SD

In-vivo biological activities: The median lethal dose (LD_{50}) of the ethanolic extract of *O. elliptica* was determined and the results revealed that the extract is considered safe up to 5000 mg/kg b.wt.; indicated by no death occurring in both phases of the experiment.

Table 4. Effects of *O. elliptica* extracts and indomethacin on carrageenan-induced rat paw edema

Group	Dose (mg/kg b.wt.)	Inhibition (%) 1 h	2 h	3 h	4 h
Indomethacin	50	63.72276	61.67004	58.01383	59.32584
Ethanol extract	250	71.10998	66.83547	60.06704	56.0206
	500	72.1997	66.94801	63.70207	64.03558
DCM fraction	250	61.75874	54.70403	50.79614	49.1573
	500	61.50532	49.89872	44.2489	44.50375
n-BuOH fraction	250	56.51292	52.27324	47.8106	45.67416
	500	57.74202	52.74589	48.32391	40.6367

Values are expressed as inhibition % (n = 6) * P< 0.05 statistically significant from the control normal inflamed group

In vivo anti-inflammatory activity: The anti-inflammatory activity of the ethanolic extract, DCM and *n*-BuOH fractions of *O. elliptica* was evaluated by carrageenan-induced rat paw oedema method and the results are displayed in Table 4. The extracts were tested at two different dose levels and indomethacin was used as the standard drug. The experimental findings indicated that all extracts produced a reduction of oedema throughout the entire period of observation (1 h to 4 h). The ethanolic extract displayed maximum effect at the first hour with the inhibition reaching 71% and 72% at 250 and 500 mg/kg b.wt, respectively, which was higher than indomethacin, exhibiting 63.7% inhibition at 50 mg/kg b.wt. The DCM fraction showed results comparable to indomethacin with % inhibitions of 61.7% and 61.5% followed by the *n*-BuOH fraction showing 56.5% and 57%, at doses of 250 and 500 mg/kg b.wt, respectively. The percentage inhibition of all the tested extracts declined slightly by the third hour, while that of indomethacin persisted. Carrageenan induced rat paw oedema is a well-studied model of acute inflammation widely used for evaluating anti-oedematous agents [25] and is typically associated with activation of the cyclo-oxygenase (COX) pathway. Indomethacin acts by blocking the two phases of oedema development i.e. blocking histamine and serotonin release within the first hour of oedema, and preventing release of some of the inflammatory mediators, via blocking the prostaglandin's action within the second phase [26]. The ethanolic extract, as well as, the DCM and *n*- BuOH fractions of *O. elliptica,* appears to follow the same pattern as indomethacin with the

inhibitory action being more pronounced at the first hour indicating a more pronounced effect on histamine and serotonin release. The significant anti-inflammatory activity observed for the ethanolic and the DCM fraction of *O. elliptica* could be attributed to a combination of the various phytochemicals detected *viz*; pentacyclic triterpenes, *β*-sitosterol glucoside and alkaloids. These results are in accordance with previous findings from the literature in which lupeol and lupeol acetate were reported to exhibit higher anti-inflammatory activity than indomethacin in rat and mouse models of inflammation [27, 28]. Lupeol was reported to act as an anti-inflammatory agent with a mechanism similar to COX-selective inhibitor drugs [29], as well as, *in-vitro* inhibition of PGE2 and tumour-necrosis factor-α [30]. Ursolic acid was as well reported to have anti-inflammatory, in addition to antioxidant and cytotoxic activities [31]. Ellipticine and 9-methoxyellipticine isolated from *O. moorei* were found to be active when tested in a pro-inflammatory gene down-regulation assay [32].

Conclusion

Cancer is considered a disease with numerous etiological factors and multiple culprits involved in its pathogenesis. It is clear that anti-inflammatory and antioxidant therapy is valuable towards early neoplastic progression and malignant transformation. Our study provides evidence for the efficacy of *O. elliptica* Labill. as a Cdc25 phosphatase inhibitor and a cytotoxic agent in treatment of breast cancer. Moreover, promising new anti-inflammatory and antioxidant activities were also established for the plant. *Ochrosia elliptica* leaves could also be considered a good source of 9-methoxyellipticine alkaloid which exhibited significant cytotoxic and antioxidant activities.

Supporting Information

Supporting information which accompanies this paper on http://www.acgpubs.org/RNP

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