Hygeia :: journal for drugs and medicines
April 2014 - September 2014
OPEN ACCESS
A half yearly scientific, international, open access journal for drugs and medicines
Research article section: Phytochemistry / Pharmacology
DOI: 10.15254/H.J.D.Med.6.2014.119

Antioxidant potential of *Cedrela odorata* stems extracts and Bio active Phytoconstituents

Khaled Rashed

Pharmacognosy Department, National Research Centre, Dokki, Giza, Egypt.

Article history: Received: 12 January 2014, revised: 4 February 2014, accepted: 17February 2014, Available online: 3April 2014

ABSTRACT

***Plan and Methodology*:** *This study evaluated antioxidant activity of Cedrela odorata stems extracts and also investigated the bioactive phytoconstituents in the bio-active extract. N-hexane, dichloromethane (DCM), ethyl acetate and methanol 80% extract were tested for free radical scavenging activity on model reaction with stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH).*

Outcome: *The results showed that dichloromethane was the most active one as antioxidant agent and phytochemical analysis of the extract revealed that it is rich with triterpenes. Chromatographic separation of that extract resulted in the isolation and identification of two triterpenic acids, oleanolic and ursolic acids and luteolin. The results may help to discover new chemical classes of natural antioxidant substances that could serve as selective agents for infectious diseases.*

Keywords: *Cedrela odorata*, stems, antioxidant effect, phytochemicals.

1. INTRODUCTION

Antioxidants are radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anemia, asthma, arthritis, inflammation, ageing process and perhaps dementias[1]. Free radical formation during the metabolism of xenobiotics is therefore an important mechanism employed by toxic agents in causing cellular damage.

Today, plant materials remain an important resource for combating illnesses, including infectious diseases and many of these plants have been investigated for novel drugs or templates for the development of new therapeutic agents[1]. There is growing interest toward natural antioxidants from herbal sources[2]. *Cedrela odorata* is a large tree from Meliaceae family and it is native to South America and the West India. The tree is known for its red, rot-resistant wood that is used to make furniture and guitars[3]. In traditional medicine, decoctions of the bark are used to treat wounds, fever, bronchitis, indigestion and other gastrointestinal ailments[4].

For Correspondence: khalednabih2015@yahoo.co.uk
Contact: 01003642233

Hygeia journal for drugs and medicines, 2229 3590, 0975 6221
Rid: J-3090-2013

Cedrela odorata essential oil from the bark showed a good antimicrobial activity[5]. The present study deals with determination of antioxidant activity of *Cedrela odorata* stems extracts and identification of the bio-active compounds of the bio-active extract.

2. MATERIALS AND METHODS

2.1. Experimental

UV/VIS: Shimadzu UV-visible recording spectrophotometer model-UV 240 (NRC, Egypt). ^{1}H-NMR and ^{13}C-NMR (Varian Unity Inova). MS (Finnigan MAT SSQ 7000, 70 ev). (Silica gel (0.063-0.200 mm for column chromatography) and Sephadex LH-20 (Pharmacia Fine Chemicals). Thin layer chromatography (TLC) F_{254} plates. Solvent mixtures, BAW (*n*-butanol: acetic acid: water 4:1:5 upper phase, 15% acetic acid: water: glacial acetic acid: 85:15).

2.2. Plant material

The stems of *Cedrela odorata* were collected from Zoo garden, Giza, Egypt in May 2011 during flowering and identified by Dr. Mohammed El-Gebaly, Department of Botany, National Research Centre (NRC) and by Mrs. Tereez Labib Consultant of Plant Taxonomy at the Ministry of Agriculture and director of Orman botanical garden, Giza, Egypt. A voucher specimen is deposited in the herbarium of Zoo garden, Giza, Egypt.

2.3. Preparation of plant extract.

The stems of *Cedrela odorata* (670 g) were extracted with n-hexane, dichloromethane (DCM), ethyl acetate and methanol 800% several times until exhaustion at room temperature by maceration method. The extracts were concentrated under reduced pressure to give 15 g, 9 g, 7.5 g and 32.5 g, respectively. Phytochemical analysis of the plant extracts were done according to that described by Yadav and Agarwal (2011)[6].

2.4. DPPH assay

The antioxidant activity of the various solvent extracts was measured according to standard protocol[7]. DPPH radical scavenging activity from the plant extract was measured by taking 100µg/ml of extract, 900µl of acetate buffer and 3 ml freshly prepared 100µM DPPH solution in methanol. Reagent blank was 1 ml buffer and 3 ml DPPH solution. The absorbance was measured after 90 min of incubation in dark at 517 nm. DPPH radical scavenging activity (%) was determined by following equation: DPPH radical scavenging: Activity (%) = A_b-A_s /A_b x 100. Where A_s - absorbance of the test sample, A_b - absorbance control reaction

2.5. *Isolation of bioactive compounds from DCM extract of Cedrela Odorata stems*

DCM extract (7 g) was subjected to silica gel column chromatography eluting with *n*-hexane, dichloromethane, ethyl acetate and methanol gradually. The fractions that showed similar thin layer chromatography (TLC) were collected and according to that three fractions were collected. Three compounds 1, 2 and 3 were isolated and identified by different spectroscopic tools (^{1}H-NMR, ^{13}C-NMR, EI-MS).

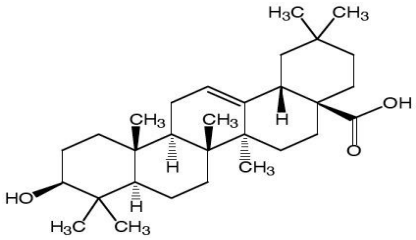
3. RESULTS AND DISCUSSION

3.1. Structure elucidation of the isolated compounds

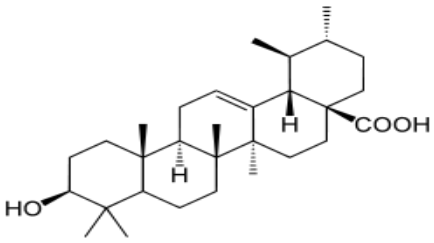
Oleanolic acid (1): 12 mg, white amorphous powder. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 5.23 (IH, t, J=3.4, H-12), 3.17 (1H, dd, J=10, 4.2 Hz, H-3), 2.74 (1H, dd, J=12.5, 4 Hz, H-18), 0.95 (3H, s, Me-23), 0.76 (3H, s, Me-24), 0.85 (3H, s, Me-25), 0.77 (3H, s, Me-26), 1.23 (3H, s, Me-27), 0.89 (3H, s, Me-29), 0.95 (3H, s, Me-30). (+) ESI-MS: m/z 455 $[M-H]^+$.

Ursolic acid (2): 10 mg, white powder. ^{1}H-NMR ($CDCl_3$, 400 MHz):δ 5.26 (IH, t, J=3.5, H-12), 3.17 (1H, dd, J=10, 4.2 Hz, H-3), 2.15 (1H, d, J=11.5 Hz, H-18), 1.92 (1H, dd, J=12.8, 4.2 Hz, H_b-22), 1.12 (1H, m, H_a-22), 1. 22 (3H, s, Me-23), 0.94 (3H, s, Me-24), 0.75 (3H, s, Me-25), 1.04 (3H, s, Me-26), 1.12 (3H, s, Me-27), 0.92 (3H, d, *J*= 6.4 Hz, Me-29), 0.89 (3H, d, J=5.8 Hz, Me-30). (+) ESI-MS: m/z 455 $[M-H]^+$.

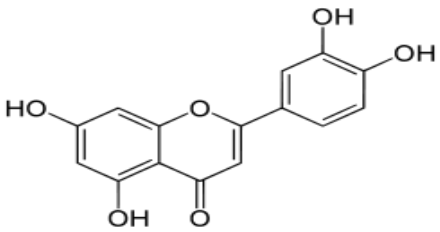
Luteolin (3): 12 mg, pale yellow crystals: UV: λ_{max} (nm) (MeOH): 254, 350, (NaOMe): 273, 411, ($AlCl_3$): 272, 330, 343, 421, ($AlCl_3$+HCl): 258, 277, 386, (NaOAc): 266, 296, 367 (NaOAc+H_3BO_3): 260, 306, 370. ^{1}H-NMR: δ 12.9 (s, 1H, 5-OH), 7.36 (d, *J*=7.9 Hz, 1H, H-6´), 7.34 (d, *J*=2 Hz, 1H, H-2´), 6.82 (d, *J*=8 Hz, 1H, H-5´), 6.64 (s, 1H, H-3), 6.42 (d, *J*=2 Hz, 1H, H-8), 6.17 (d, *J*=2 Hz, 1H, H-6). EI-MS: *m/z*: 286.



1: Oleanolic acid



2: Ursolic acid



3: Luteolin

Figure. 1. Chemical structures of the compounds isolated from *Cedrela odorata* stems

3.2. Identification of the isolated compounds

Compound 1 (Oleanolic acid), compound 2 (ursolic acid) were monitored by TLC, and the spot of each compound was detected by heating the plates at 110^{o}C after spraying with p-anisaldehyde–sulfuric acid, also spectral data were in agreement with published data[8]. Compound 3 (luteolin) was isolated as a deep purple spot and with spraying with $AlCl_3$, it gave a yellow fluorescence colour under UV light; UV spectral data confirm that it is a flavone with free OH groups at positions 3', 4', and 5, 7; ^{1}H-NMR and MS are very similar to the characteristics of luteolin reported by Saeidnia et al. (2009)[9].

The present study was focused on the evaluation of anti-oxidant activity of *Cedrela odorata* stems extracts where DCM extract showed a significant anti-oxidant activity (table 1). Also we investigated the presence of phytochemicals in the extracts of *Cedrela odorata* stems extracts and pphytoconstituents are shown in table 2. *Cedrela odorata* DCM extract has a different bioactive components as flavonoids and triterpenes (table 2), *Cedrela odorata* extract is rich with flavonoids and triterpenes which are present in considerable amounts in the extract, these compounds have good antioxidant effect[10].

The DPPH radical scavenging activity of *Cedrela odorata* stems extracts were compared with that of known natural green tea (table 1) where DCM extract showed a significant antioxidant potential (85.45%) and the other extracts were less active as antioxidant agents. As revealed by Ahmadi et al. (2007)[11], DPPH method measures the ability of antioxidants present in scavenging the hydrophilic free radicals. In line to this theory, ethyl acetate extract has better ability in scavenge hydrophilic free radicals as compared to other *Cedrela odorata* stems extracts that might due to the presence of hydrophilic antioxidants.

Furthermore, the high antioxidant activity could be due to the increased in hydroxyl groups or antioxidant compounds found particularly in the *Cedrela odorata* stems DCM extract. DCM extract is very rich with flavonoids and triterpenes. Flavonoids show antioxidant activity and their effects on human nutrition and health are considerable.

The mechanisms of action of flavonoids are through scavenging or chelating process[12]. The highest level of radical scavenging properties at low concentrations of flavonoids exhibits quercetin and in the following order luteolin, rhamnetin, isorhamnetin and apigenin[12]. The extract of *Salvia macrochlamys* was tested in five different methods for potential antioxidant activity consisting of free radical scavenging activity by DPPH, by β-caroten–linoleic acid and by superoxide anion radical scavenging activity and the extract showed a good antioxidant effect at 12.5-25 μg/mL concentrations and this activity is due the triterpenes and flavonoids isolated from the extract[13].

Table. 1. Antioxidant activity of *Cedrela odorata* stems extracts

Extracts	*Concentration (%)*	*DPPH free radical scavenging effect (%)*
Green tea extract	1%	96.41%
N-hexane extract	0.1%	26.94%
Dichloromethane extract	0.1%	85.45%
Ethyl acetate extract	0.1%	72.75%
Methanol extract	0.1%	66.25%

Table. 2. Phytoconstituents of *Cedrela odorata* stems extracts

Chemical Constituents	N-hexane	Dichloromethane	Ethyl acetate	Methanol
Carbohydrates and/or glycosides	-	-	+	+
Tannins				
a. Condensed tannins	-	-	+	+
b. Hydrolysable tannins	-	-	+	+
Alkaloids and/or nitrogenous bases	-	-	-	-
Flavonoids	-	+	+	+
Sterols and/or triterpenes	+	++	+	+
Saponins	-	-	-	-
Coumarins	-	-	-	-

(+) denotes the presence of the constituents, (-) denotes the absence of the constituents

4. CONCLUSION

The presented results indicate that antioxidant potential of *Cedrela odorata* stems DCM extract is due the presence of bio-active phytoconstituents as phenolic compounds (flavonoids) and triterpenes and these results also endorsed the ethnobotanical use of this plant from the collected territory due to presence of various chemicals.

Conflict of interest

There is no conflict of interest associated with the authors of this paper.

REFERENCES

1. Oke, J.M., Hamburger, M.O.. Screening of Some Nigerian Medicinal Plants for antioxidant activity using 2,2, Diphenyl- Picryl-Hydrazyl Radical. *African Journal of Biological Research* **2002**; 5: 77 – 79.
2. Larson, R.A. The antioxidants of higher plants. *Phytochemistry* **1998**; 27(4): 969-978.
3. Zuchowski, W. A guide to Tropical Plants of Costa Rica, Zone Tropical Publishing, Miami, Floride, **2005**.
4. Morton, J.F. Atlas of Medicinal Plants of Middle America, vol. I, Charles C. Thomas, Publisher, Springfield, Illinois, **1981.**

5. Heather, E.V., Jessika, A.T., William, A.H., William, N.S. Chemical composition and antimicrobial activity of the bark essential oil of *Cedrela odorata* from Monteverde, Costa Rica. *Der Pharma Chemica* **2009**; 1 (2):14-18.
6. Yadav, R.N.S., Agarwala, M. Phytochemical analysis of some medicinal plants. *Journal of Phytology* 2011; 3(12): 10-14.
7. Saha, M.R., Hasana, S.M.R., Aktera, R., Hossaina, M.M., Alam, M.S., Alam, M.A., Mazumder, M.E.H *In-vitro* free radical scavenging activity of methanol extract of the leaves of *Mimusops Elengi Linn*. *Banglesh Journal of Veterinary Medicine* **2008**; 6 (2):197–202.
8. Seebacher, W., Simic, N, Weis, R., Saf, R., Kunert, O. *Magnetic Resonance Chemistry* **2003**;41:636–638.
9. Saeidnia, S., N. Yassa, R. Rezaeipoor, A. Shafiee, A.R. Gohari, M. Kamalinejad and S. Goodarzy. Immunosuppressive principles from *Achillea talagonica*, an endemic species of Iran. *DARU* **2009**; 17 (1): 37-41.
10. Hopia L., Heinonen S. Antioxidant activity of flavonol aglycones and their glycosides in methyl linoleate. *Journal of the American Oil Chemistry s' Society.* **1999**; 76 (1): 139-144.
11. Ahmadi, F., Kadivar, M., Shahedi, M. Antioxidant activity of *Kelussia odoratissima Mozaff.* in model and food systems". *Food Chemistry* **2007**; 105:57-64.
12. Kessler, M., Ubeaud, G., Jung, L. Anti- and pro-oxidant activity of rutin and quercetin derivatives. *Journal of Pharmacy and Pharmacology* **2003**; 55: 131-142.
13. Gülaçtı, T., Abdülselam, E., Ufuk, K., Mehmet, Ö., Ayhan, U. *ARKIVOC* , **2007**; 7: 195-208