The Extraction and Partial Purification of Wedelolactone from Local *Eclipta alba* Plant

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Abstract:
Eclipta alba is a weed growing in damp, moist puddles distributed in the tropical and subtropical regions, so the most weight of the plant is water, which reached in to 90%. The extraction method by using different solvents (Methanol, Ethanol, Hexane and Aqueous) showed, the best yield with methanol reached to 76%, and the yield decreased with ethanol and hexane reached 55% and 53% respectively, while the minimum yield observed with aqueous hot and cold extraction reached 11% and 5% respectively. The phytochemical compound characterization showed the compounds (Coumarines , Flavones ,Volatile Oil ,Tannins , Saponines , Glycosides ,Carbohydrates ,Alkaloids , Resins) with different percentage. The thin layer chromatography detection for wedelolactone showed appositive result according to the appearance of purple to a violet color under UV .light, and the value of R_F was reached to 0.56 cm .The purification method showed one main peak between the fractions (7-22). The melting point for wedelolactone was 328. The HPLC result showed compatibility between both sample and standard wedelolactone in shape, and the retention time for the sample was 2.157 which is closely related with the standard one 2.163 , and the concentration of the wedelolactone was 99.27
Key words: *Ecliptaalba* ,wedelolactone , wedelolactone from *Eclipta alba*.

الاستخلاص والتنقيه الجزئيه لماده الWedelolactone من النبات المحلي العرندس

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الخلاصة:
نبات *Eclipta alba* العشبي ينمو في البرك والمناطق الاستوائيه وشبه الاستوائيه الرطبه لذا معظم الوزن من النبات هو الماء ،اذ بلغت الرطوبه 90% .وتم استخلاص المركبات الفعاله في النبات باستخدام الميثانول والايثانول والهكسان والمائي . وافضل نتائج تم الحصول عليها باستخدام الميثانول وصلت نسبه الاستخلاص به 76% والايثانول الى 55% والهكسان وصلت الى 53% بينما المذيب المائي الساخن 11% والباردالى 5% .كذلك تم توصيف المركبات الكيميائيه للنبات فوجد (الكومارينات، الفلافونات، الفينولات، الزيوت الطياره، التانينات، صابونيات، الكلايكوسيدات، كاربوهيدرات، القلويدات، الراتنجات) ولكن بنسب مختلفه .تم الكشف عن ال Wedelolactone باستخدام كروموتوكرافي الطبقه الرقيقه (TLC.) اظهرت نتائج وجود اللون البنفسجي الى الارجواني تحت ال.UV وحسبت قيمه الـRF بلغت 0.56سم .اظهرت التنقيه اعلى قراءه في الـfractions مابين(7−22).وكانت درجه الانصهارلل wedelolactone 328 درجه ،اظهرت نتائج التحليل للـHLPC وجود توافق بين كل من العينه المنقات جزئيا والعينه القياسيه من خلال زمن الاحتجاز لكل منهما اذ بلغ 2.163 للقيمه القياسيه بينما للعينه المنقاة جزئيا 2.157 ومنها تم قياس تركيزالـWedelolactone اذ بلغ 99.27 .

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Introduction:

Eclipta alba is a weed /herb growing in damp, moist puddles distributed in the tropical and subtropical regions of the world. So besides ethnobotanicalevidence, it can be hypothesized that plants which survive in media rich in microbes most likely be possessing antimicrobial principles [1]. Main active principles consist of coumestans like wedelolactone, desmethylwedelolactone [2], furanocoumarins, oleanane&taraxastane glycosides [3]. Various biological activities are possessed by *E. alba*, such as memory disorders treatment, general tonic, edema, fevers and rheumatic joint pains treatment, digestion, hepatitis, enlarged spleen, antioxidant activity and skin disorders [4, 5, 6]. Wedelolactone is active principle compound of liver disorder treating drug [2]. It also exhibits Trypsin inhibitory effect [7, 8]. Suppresses LPS-induced caspase-11 expression in cultured cells by directly inhibiting the IKK complex [9]. Treatment of cirrhosis of the liver and infectious hepatitis [10]. However, up to date, research has been done to investigate various pharmacological activities and antimicrobial activity of only crude extracts of this traditionally used herb [11].This study was aimed to obtained the highly purified wedelolactone from local *Eclipta alba*

Materials and methods:

Plant collection:

The aerial parts of *Eclipta alba* (Asteraceae) were collected locally from different places nears the Tigris river in Bagdad, during November 2011 to January 2012. The collected plants were cleaned with distilled water. The weights of the aerial plants parts were measured.

Plant drying:

The clean collected plants were left at room temperature at (22-25) °C for 3-4 weeks for drying, then grinding by electric Blander to convert the dried plants into powder (pdr).

Estimation of humidity:

The humidity percentage of the aerial plants was estimated according to the following equation [12].

$$\text{Humidity \%} = \frac{\text{Wet weight} - \text{dried weight}}{\text{Wet weight}} \times 100$$

Extraction of crude wedelolactone:

The soxhlet thumble was filled with twenty grams of dried plant powder , and put in the soxhlet apparatus with 180 ml from one of different solvents which were tested (Hexane, Ethanol absolute, Methanol absolute, Aqueous) at 50°C for 36 hours[1].

Filtration and concentration the extracted sample:

The extracted sample were filtered through filter paper (what man no.1) the filtered sample was concentrated by using rotary evaporator at a temperature varies according to the solvent which was used then the yields were kept in container at 4°C until further use.

Characterization of phytochemical compound.

Coumarines test:-

The test was done according to the methods followed by Thenmozhi *et al*., method [13]. Four drops of concentrated sample were put near the adage of aluminum oxide plate of thin layer chromatography(TLC)plate , then put the TLC plate in the jar with three different solvents system Toluene :Acetone :Formic acid (11:6:1),which act as mobile phase, then the transferred spot was tested under UV. Light, the appearance of purple to violet color was indicated the coumarines were presence in the plant.

Flavones test:-

The test was done according to method of Jaffer *et al.,* [14]. A-quantity of 10 g of the powdered explants was macerated in 95% ethanol than filtered with filter paper (what man no.1) . B-aliquot of 10ml of 50% ethanol was added to 10ml of 50% aqueous KOH the solution was mixed with solution b appearance of yellow color indicated positive result.

Volatile Oils test:

The method was depended on procedure described by Jammutavi [15]. Three milliliter of plant extract after filtration was put under UV. Light, the appearance of pinkish color was indicated the presence of volatile oil.

Tannins test:

The procedure was depended on Shami method [16]. Tow milliliter of plant extract sample after filtration were added to 1% of lead acetate, the appearance of precipitant gel was indicated to a positive result .

Phenol compound test

The procedure was depended to the methods described by Thenmozhi *et al*., method (13). Tow milliliter of plant extract after filtration were added to 1% of ferric chloride , the appearance of dark blue color was indicated to the presence of phenol compound.

Saponines test:

The method was done according to the Shami method [16]. One milliliter of mercuric chloride (1%) was added to 2ml of plant extract after filtration the observation of white precipitate was indicated to a positive result.

Glycosides test:

The procedure was done according to the shahatt method [17]. By addition of few drops from Kedde reagent to 3ml of plant extract after filtration. The appearance of blue–purple color is indicated to a positive result.

Alkaloids test:

The method was done according to the shahatt method [17]. A few drops of modified Dragendroffs reagent to 5ml of plant extract after filtration. The appearance of red dish orange color is indicated to a positive result.

Carbohydrates test:

The test was done according to the Thenmozhi *et al*., method [13]. Five milliliter of plant extract after filtration were treated with 5ml of Fehling solution , then kept in water bath at (100)°C, the formation of yellow precipitate that positive result .

Resins test:

The test was done according to the Shami method [16]. Ten milliliter of acidified diluted HCL solution 4% was added to 5ml of plant extract after filtration the formation of turbidity that positive result.

Partial purification of crude extract

Activation and preparation of silica gel

The test was done according to method of Punima *et al.,* method [18] twenty gram of silica gel powder were activated by using oven with 150°c for one hour, cooled for few minute and suspended with 100 ml of methanol with stirring, then this suspension was put slowly in glass column (1×80) cm until settling, and the final silica length was 50 cm, and setting the flow rate with methanol in to 3ml /5min. [14].

Preparation of mobile phase:

The mobile phase was prepared from two different organic solvent which are methanol and chloroform (70:30) [1].

Fractions collection:

The fractions were collected after the sample was put in the prepared column.

Spectrophotometer measurement:

The fraction was measured by spectrophotometer with UV. Wave Length (351)nm.

Techniques:

Thin layer chromatography

The aluminum sheet was marked 1 cm from the bottom and the Spots were made from crud and partial purified extract. Then the plate was soaked gently in the TLC jar contain Toluene: Acetone: Formic acid (11: 6: 1) as a mobile phase. The solvents were moved until they reached upper adage. Then the plate was removed from the jar and allowed to dry, the spots were noted and the Rf value was calculated according the following equation:

$$RF = \frac{\text{Distance of spots sample movement}}{\text{Distance of spots solvents movement}}$$

High Performance Liquid Chromatography (HPLC):

The partial purified and standard sample was measured by HPLC according to the Punima *et al.,* method [19]. At 351 nm and concentration of the active material (wedelolactone) was measured according to the following equation.

$$\text{Assay concentration \%} = \frac{\text{AUC (test)}}{\text{AUC (standard)}} \times 100$$

AUC: Area under curve

Melting point:

The melting point of the partial purified sample was measured according to Jammutavi procedure [15].

Result and discussion

The result of wet quantity measurement for the aerial parts of plant was 90% ,so the most weight of the plant is water that is may be due to the environmental condition were the plant where grown. The extraction method by using different solvents (Methanol , Ethanol , Hexane and Aqueous) showed ,the best solvents could extracted the active material was Methanol then Ethanol and Hexane, according to the percentage of the extracted material which reached 76% , 55% ,and 53% respectively ,while the hot Aqueous extraction percentage showed 11% than cold one which reached 5% . The characterization of phytochemical compound of the crud extract showed there were different compounds (Coumarines , Flavones ,Volatile Oil ,Tannins , Saponines , Glycosides ,Carbohydrates ,Alkaloids , Resins) But in different percentage and this result agreed with Thenmozhi *et al*. [13] Table 1**.**

Table 1- The characterization of phytochemical compound

No	Test	Color Regent	Result
1	Coumarines	Purple (TLC)	++
2	Flavones	Yellow	++
3	Volatiles oil	Pinkish	++
4	Tannins	Gel precipitate	++
5	phenolic compound	Blue color	++
6	Saponines	White precipitate	+
7	Glycosides	Purple	+
8	Carbohydrate	Yellow precipitate	+
9	Alkaloids	Orange – red	+
10	Resins	Turbidity	+

Strong: ++ weak: +

The thin layer chromatography detection for active material (wedelolactone) showed appositive result as shown in Figure 1

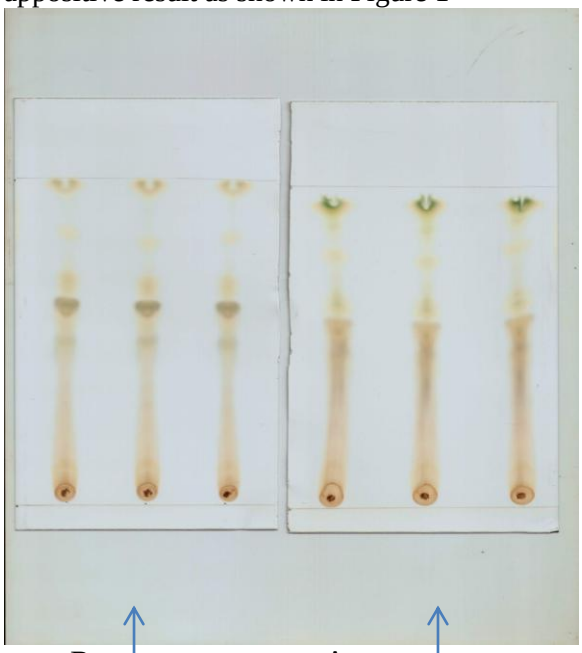

Figure 1- Thin layer chromatography sheets for active material (wedelolactone)
A: transfer crude sample extract in TLC.
B: transfer partial purified sample in TLC

The RF. Value was 0.56 for the tested sample spot as shown in the following equation

$$R_F = \frac{9}{16} = 0.56 \text{ cm}$$

And when the sample sheet was tested under UV .light, the purple to a violet color was appeared from the sample spot .This result agreed with Thenmozhi *et al*. [13]. But not agreed with Sunita *etal* .,[1], this disagreement may be due to the differentiation between the plant species among the countries, and the environmental condition.

The result of the purification method showed there was one main peak occur between the fractions (7-22) (Figure 2), the fractions were collected and concentrated for further applications.

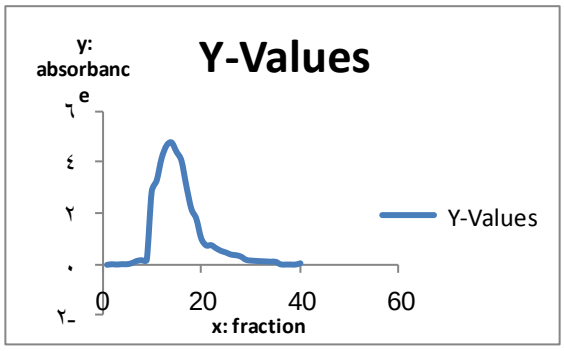

Figure2- showed the absorbance result at (351) nm for partial purificationsamplein (1X80) Cm of Silica gel column with flow rate 3 ml/5min.

The active material (wedelolactone) in the concentrated sample was checked with standard wedelolactone by HPLC, the result showed there were compatibility between both sample and standard in shape, and the retention time for the sample was 2.157 which is closely related with the standard one 2.163 (Figure 3 ,4)

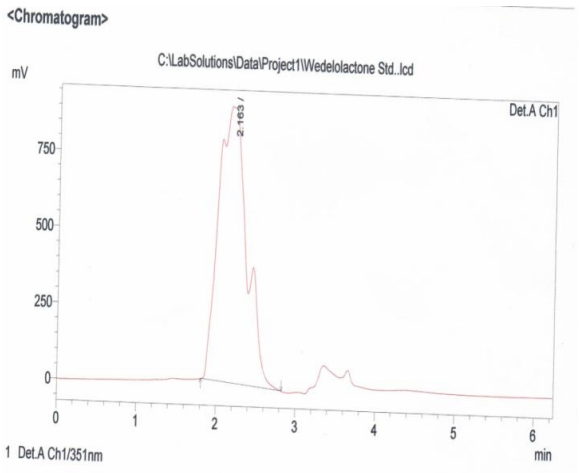

Figure3- HPLC figure for standard wedelolactone

Peak Total: Detector A ch1 351nm

Peak π	Ret. Time	Area	Height	Area %
1	2.163	22519981	904556	100.000
Total		22519981	904556	100.000

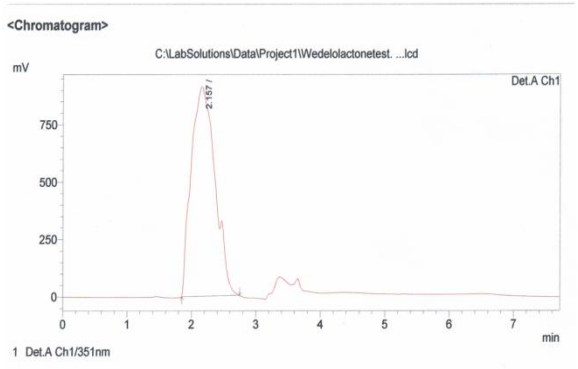

Figure 4- HPLC figure for sample wedelolactone

Peak Total: Detector a ch1 351nm

Peak π	Ret. Time	Area	Height	Area %
1	2.157	22356727	892038	100.000
Total		22356727	892038	100.000

And the concentration of the wedelolactonewas 99.27 according to the following equation.

$$\text{Conc.} = \frac{22356727}{22519981} \times 100 = 99.2\%$$

The Figure 4, showed the efficient of purification method to purify the sample .This result confirmed also according to the result of the melting point which was 328, and this result agreed with Jammutavi result [15].

Conclusion:

Eclipta alba is a weed; the most weight of the plant is water, which reached in to 90%. The best solvents which could extracted the active material were Methanol , Ethanol , Hexane , hot Aqueous and cold one respectively .The characterization of phytochemical compound in the crud extract showed there were (Coumarines , Flavones ,Volatile Oil ,Tannins , Saponines , Glycosides ,Carbohydrates ,Alkalaoids , Resins) But in different percentage. The thin layer chromatography detection for wedelolactone showed appositive result. The purification method showed the efficiency due to the result of the melting point and the HPLC result according to the compatibility between both sample and standard wedelolactone.

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