Original Article

Physicochemical study of the homoeopathic drug, *Blatta* orientalis

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Abstract

Introduction: *Blatta orientalis* or oriental cockroach, also known as water bug or black beetle, belongs to class insecta and family Blattidae. In Homoeopathy, medicine prepared from it is used in a number of ailments, especially in the treatment of asthma. **Materials and Methods:** The physicochemical study includes evaluation of different parameters, namely foreign matter, moisture content, total ash, water-soluble and acid-insoluble ash values, water and ethanol extracts of the raw drug. Physicochemical studies, namely organoleptic specifications, sediment, specific gravity determination, total solids, pH measurement, thin-layer chromatography (TLC), ultraviolet-visible spectra and alcohol content have also standardised and presented for both in-house and commercial mother tinctures (finished products). **Results:** The study indicates that the values of the preliminary parameters of this drug are quite different from the ranges reported for plant drugs. TLC study confirms the complexity of the composition of the prepared drug. **Conclusion:** The physicochemical data of the drug, *B. orientalis,* may serve as pharmacopoeial standard for authentication, quality assurance and quality control process of the commercially available drug.

Keywords: Asthma, Blatta orientalis, Homoeopathy, Physicochemical

INTRODUCTION

It is beyond any debate that nature is the single most important source of bioactive compounds as almost all organic medicines presently available are either originated or inspired from natural products.^[1-15] Among natural products, medicinal plants have caught great attention from drug discovery community.^[16-19] However, several medicines which are derived from animals in general or their secretions or products, such as different kinds of worms, lice, insects, beetles, flies, crabs, toads and snakes are also used in Homoeopathy for various ailments. [20-26] Homoeopathy rightly recognised the potential of animal kingdom for medicinal purpose from its origin and among them, Blatta orientalis is quite distinctive.[27,28] The medicinal activity of B. orientalis was accidently discovered when B. orientalis-contaminated food (tea) relieved a patient from asthma by considering the 'Law of Similars'. In Homoeopathy, B. orientalis mother tincture and its dilutions are prescribed to treat asthma, bronchitis, cough and dyspnoea.^[29]

B. orientalis is regarded as a harmful pest responsible for a number of diseases, including dysentery, food poisoning and diarrhoea.^[30-34] The mother tincture of

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Quick Response Code:	Website: www.ijrh.org	
	DOI: 10.4103/ijrh.ijrh_24_18	

B. orientalis has been found to possess anti-asthmatic activity against acetylcholine and histamine aerosol-induced bronchospasm in guinea pigs.^[35] The mother tincture has also been reported to possess anti-anaphylactic activity against passive as well as anaphylaxis models in rats.^[35] Haemolymph of *B. orientalis* had shown *in vitro* antibacterial activity against Staphylococcus aureus, Proteus mirabilis, Salmonella typhi, Pseudomonas aeruginosa and Escherichia coli.^[36] The reported chemical constituents of haemolymph of B. orientalis are triazoles, thiophenes, secondary sulphonamide, vinyl halides, sulphinic acid, secondary amide, bromo compounds, cyclopeptane, aldehyde/ketones group and methylene groups.^[36] Considering its medicinal effect as potential anti-asthmatic drug in Homoeopathy, the present study was carried out to standardise the raw drug and homoeopathic formulation (mother tincture) of B. orientalis.

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> > Received: 07.04.2018; Accepted: 11.09.2018

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How to cite this article: Biswas B, Jhansi S, Potu R, Patel S, Nagaraju M, Arya R, *et al.* Physicochemical study of the homoeopathic drug, *Blatta orientalis.* Indian J Res Homoeopathy 2018;12:125-31.

The physicochemical data presented in this research article may also be used as pharmacopoeial standard to ensure the quality of commercial samples. This work upgraded already published monograph of *B. orientalis* at HPI.^[37]

MATERIALS AND METHODS

Collection of B. orientalis

Live B. orientalis was collected from household manholes and corners of buildings by using sterile surgical gloves which were duly identified and authenticated with the help of Department of Zoology, Osmania University, Hyderabad.

Description of Blatta orientalis

Drug was prepared from whole live B. orientalis Linn. (synonym: Blatta lucifuga Poda; Blatta badia Saussure; Blatta castanea Blanchard; Family: Blattidae). B. orientalis is a common cockroach in India, where it inhabits human dwellings and damps moist corners. It is a dictyopteris insect, with an elongated oval rather flat body, from 18 to 30 mm in length, of a red or brown-red colour, which becomes paler under belly. Mouth parts are biting type, mandibles are strong and toothed. The prothorax is smooth, shining, with two large brown spots. In the male, the elytra reach beyond the belly. In the females, they are little shorter. The wings are striate and reticular, of the length of elytra. The antennae are filiform, longer than the body and exhibit at their base is small yellowish point. The feet are provided with black prickles and terminate in tarsus with five articulations and a pair of claws with pulvilus. In B. orientalis, the dorso plunal line of the abdomen is contained in the narrow, unfolded lateral membrane uniting the paratergites and the large ventral plates. In the male cockroach, the ventral plate of the ninth segment bears a pair of the styles. Genital segments of the female are almost entirely concealed within the seventh segment. Anal cerci (a pair) are present in both sexes.

Physicochemical studies

Physicochemical studies, namely foreign matter, moisture content, total ash, water-soluble and acid-insoluble ash values, water and ethanol extracts were done using whole body of B. orientalis in accordance with the procedure described by Homoeopathic Pharmacopoeia of India (HPI). For thin-layer chromatography (TLC), aluminium plates, pre-coated silica gel and fluorescent indicator 60F-254 TM of 0.25-mm thickness manufactured by Merck were used. The spots were detected by ultraviolet (UV) lights having wavelengths 366 nm and 254 nm.

Experimental procedures

Loss on drying

Around 2 g of air-dried raw drug was kept in a well-ventilated oven for 3 h at 105°C. After removing the drug from the oven, it was allowed to cool to room temperature by keeping the dried raw drug in a desiccator for 20 min. Then, it was again weighed. The loss on drying (LOD) (moisture content) in percentage was calculated as follows:

- Weight of emptying the Petri dish = W_{empty}
- Weight of the Petri dish with air-dried raw drug = W_{+wet}
- Weight of the air-dried raw drug = $w_{+wet} w_{empty} = w_{wet}$ •
- Weight of the oven-dried (105°C) raw $drug = w_{+dry} - w_{empty} = w_{dry}$

• % LOD =
$$\left[\frac{\left(w_{wet} - w_{dry}\right)}{w_{wet}}\right]$$
 * 100.

Preparation of mother tincture (ϕ)

As directed in HPI, the mother tincture was prepared according to the method described at HPI.^[37]

Preparation of potencies

The solid potencies (f) were prepared according to HPI.^[37]

Extractive values

Around 2.0 g of raw drug was weighed (moisture content was taken into account). To it, 50 mL of EtOH was added and kept for 24 h at room temperature. After filtering it, 10 mL was taken and evaporated on a water bath. Thereafter, it was heated at 105°C in a well-ventilated oven till constant weight was achieved. The above experiment was performed twice and an average value was reported. The ethanol extract value was calculated as follows.

- Drug weight = w_{drug} (moisture content was subtracted to calculate the drug weight)
- Empty beaker weight = w_{empty}
- Beaker + dried extract weight = w_{+drug}

% EtOH extract =
$$\left[\frac{\left\{\left(w_{empty} - w_{+drug}\right)\right\}}{w_{drug} / 5}\right] * 100 = \% W/W.$$

In a closely related experiment, water extract value was determined using 50 mL of water instead of ethanol.

Ash values

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Determination of total ash value

Around 2.0 g of raw drug was weighed (moisture content was taken into account) in a thermally resistant previously weighed crucible. The crucible along with its content is heated to 450°C for 30 min. The crucible was cooled in a dedicator for 15 min and weighed. This procedure was repeated till constant weight is obtained.

Then, the percentage of total ash was calculated with reference to the drug weight.

- Empty crucible weight = w_{empty}
- •
- Drug weight = w_{drug} Crucible + ash weight = w_{+ash}
- Total ash= $w_{+ash} w_{empty} = w_{ash}$

• % Total ash =
$$\left\lfloor \frac{w_{ash}}{w_{drug}} \right\rfloor$$
 * 100 = % w/w

The above experiment was performed twice and an average value was reported.

Determination of acid-insoluble ash value

The ash obtained as directed under total ash value was boiled with 25 ml of 10% HCl for 5 min. The insoluble matter was collected on an ash-less filter paper, washed with hot water, ignited for 15 min at a temperature about 450°C. The crucible was cooled in a desiccator for 15 min and weighed. The heating was repeated till a constant weight is reached. The above experiment was performed twice and an average value was reported.

- Empty crucible weight = w_{empty}
- Drug weight = W_{drug}
- Crucible + acid-insoluble ash weight = $W_{+acid(i)ash}$
- Acid-insoluble ash = $w_{+acid(i)ash} w_{empty}$

• % acid-insoluble ash =
$$\left[\frac{\left\{w_{+acid(i)ash} - w_{empty}\right\}}{w_{drug}}\right] * 100 =$$

%w/w.

Determination of water-soluble ash value

The total ash obtained was boiled with 25 ml of water for 5 min. The insoluble matter was collected on an ash-less filter paper, washed with hot water and ignited for 15 min at a temperature about 450°C. The crucible was cooled in a dedicator for 15 min and weighed, repeated for constant value. The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water-soluble ash. The percentage of water-soluble ash was calculated as follows. The above experiment was performed twice and an average value was reported

- Empty crucible weight = w_{empty}
- Drug weight = W_{drug}
- Crucible + water-insoluble ash weight = $W_{+\text{water}(i)ash}$
- Water-insoluble ash = $W_{+water(i)ash} W_{empty}$
- Water-soluble ash = Total ash Water-insoluble ash = $W_{ash} - W_{+water(i)ash} = W_{water(s)}$

• % Water-soluble ash=
$$\left[\frac{w_{water(s)}}{w_{drug}}\right]$$
 * 100 = % w/w.

Determination of total solids

A 10 mL of mother tincture of the drug was heated on a water bath to remove the alcohol. After that, the water content was removed by heating it inside a well-ventilated oven. The sample was cooled in a desiccator for 15 min and weighed. The heating process was repeated till constant value is reached. The above experiment was performed twice and an average value was reported.

- Empty beaker weight = w_{empty} Beaker + dried extract weight = $w_{+dried drug}$
- Total solids= $w_{+dried drug} w_{empty}$

• %Total solids=
$$\left[\frac{\left\{\left(w_{+dried \, drug} - w_{empty}\right)\right\}}{drug \, volume}\right]$$
 * 100 = % w/v

Specifically in this case:

• %Total solids=
$$\left[\frac{\left\{\left(w_{+\text{dried drug}} - w_{\text{empty}}\right)\right\}}{10}\right] * 100 = \% \text{W/v}.$$

Weight per mL

10 mL of the mother tincture was weighed and the weight was divided by 10 to get the data.

pН

The pH was determined by a digital pH meter. The pH of the samples (authentic and commercial) was recorded only after calibration using buffer solutions each time.

 λ_{max}

The measurement was made by diluting the mother tincture by ~100 times. The diluting solvent is same as the mother tincture solvent system.

High-performance thin-layer chromatography

Around 25 mL of the mother tincture was heated on a water bath to remove alcohol. The residue thus obtained was extracted by three 20 mL portions of chloroform. The chloroform extract was then concentrated to ~ 2 mL by heating on a water bath. This concentrated extract was used to carry out high-performance TLC (HPTLC) on pre-coated silica gel aluminium plate 60F-254 of 0.25 mm thickness manufactured by Merck, using 9:1 CHCl,:CH,OH mixture as the solvent system. The spots were detected by UV lights with $\lambda_{\text{max}} = 254$ nm and 366 nm. Furthermore, the spots were detected under visible (Vis) light via derivatisation using anisaldehyde-sulphuric acid stain.

RESULTS AND DISCUSSION

The results of the physicochemical study of the raw drug are summarised in Table 1. The LOD was found to be 76.85% which is important to gauge the amount of raw wet drug needed for the mother tincture. Furthermore, the value is quite high compared to the plant-based drug. This is quite expected considering the nature of the raw drug. Besides LOD, the ash values (particularly total ash) are considerably low compared to corresponding plant based drugs. As acid-insoluble and water-soluble ash values do not differ much from the total ash value, it could be argued that the ash value measurements could

Table 1: Raw drug parameters			
Parameters	Value		
Foreign matter	≤0.1% w/w		
Moisture content (LOD at 105°C)	≤76.85% w/w		
Total ash	≤1.55% w/w		
Acid-insoluble ash	\leq 0.35% w/w		
Water-soluble ash	\leq 0.83% w/w		
Extractive values			
a. Alcohol	≤16.35% w/w		
b. Distilled water	≤23.53% w/w		
LOS: Loss on drying			

effectively be used for approximate measurements of the heavy and alkali metals. Considerably high values of the water and ethanol extracts indicated that the raw drug has considerable amount of polar constituents.

In Table 2 (column 2), the parameters of the authentic finished product (mother tincture) have been given including the organoleptic profile. As the total solid value is considerably high, it is fair to infer that the chosen solvent system for mother tincture preparation is quite effective to extract active pharmaceutical ingredients from the raw drug. The pH of the mother tincture is close to the neutral pH. This measurement indicates that lower potencies are indeed safe for oral administration. The UV-spectra of the mother tincture showed absorptions in far UV region, indicating that highly conjugated or complex metallic complexes could be absent in the finished products. HPTLC study was performed on the chloroform extract of the mother tincture. Interestingly, spot at baseline for pure mother tincture indicates that the drug contains considerable amount of highly polar constituents. Thus, further analytical studies could be undertaken to resolve the spot. Considering the rules set by HPI, the R_s values have been solely calculated for chloroform extract of the mother tincture.

Comparative study

The study was further extended towards a comparative study of our in-house, authentic mother tincture with a commercial mother tincture.

The data of the comparative study have been summarised in Table 2. The data clearly showed that the characteristics of the commercial mother tincture are quite different from the authentic in-house mother tincture prepared in accordance with the procedure set by HPI. This comparative study is quite important as this study is first of its kind. From the data, it is quite evident that the two samples differ considerably in terms of organoleptic data, which is considered as the most preliminary data related to quality assurance. Even though sediments are absent in both the cases, this parameter solely cannot be used to authenticate. Surprisingly, total solid is quite high in case of commercial sample. This might be due to the inclusion of foreign matters during the preparation of the mother tincture. The lower value of specific gravity could be attributed to the presence of low-density components. Even though pH of the samples seems quite comparable, this is not quite the case as $pH = -log_{10}[H+]$. Using this equation, the [H+] concentration authentic sample is found to be ~ 1.5 times more than that of the commercial one. The pH value is not only important for safe oral administration but also is important for bioavailability and bioequivalence of orally administrated drugs. UV-Vis measurements showed that the samples have different λ_{max} values. Even though identical UV-Vis measurements could not be directly translated to the authenticity difference in spectra certainly indicated that the samples possess different chemical composition. In order to verify this, we explored a comparison of the chemical composition of the samples by HPTLC [Figure 1]. From the HPTLC plates, it is quite evident that the compositions of the samples are appreciably different. Considering the results,

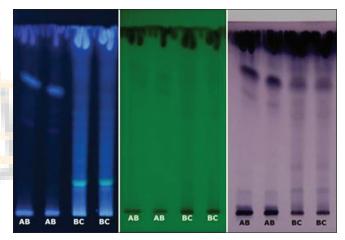


Figure 1: AB: Authentic in-house; BC: Commercial. Left: 254 nm, Middle: 366 nm, anisaldehyde-sulphuric acid stain

Table 2: Finished product parameters			
Parameter	Authentic	Commercial	
Organoleptic parameters			
a. Appearance	Clear, non-viscous	Clear, non-viscous	
b. Colour	Sunset yellow	Brown	
c. Odour	Sweet	Foul	
Sediments	Absent	Absent	
Weight per mL	≥0.89 g	≥0.81 g	
Total solids	≤0.98% w/v	≤2.07% w/v	
Alcohol content	87%-91% v/v	96% v/v*	
pH	6.53	6.74	
λ _{max}	227.47 and 265.93 nm	227.61 and 275.82 nm	
HPTLC	R,		
UV 254 nm	0.66, 0.71 (all black)	-	
UV 366 nm	0.44, 0.66 (all blue)	0.17 (yellow)	
Under visible region after derivatising with anisaldehyde sulphuric acid $R_{\rm f}$ values	0.66, 0.71 (all purple)	0.11, 0.26, 0.54, 0.71, 0.93 (all purple)	

*Reported at the label of the sample. HPTLC: High-performance thin-layer chromatography, UV: Ultraviolet

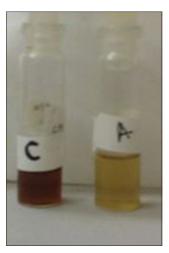


Figure 2: Authentic in-house (A) and commercial (C) mother tincture

we could infer that the commercial differs substantially from the authentic sample [Figure 2]. However, the origin of this disparity could not be ascertained without further study.

CONCLUSION

This novel study provides the physicochemical standard of the homoeopathic drug *B. orientalis*. Hence, the measured parameters furnished standards of the drug. These could be used for quality control and quality assurance of the drug, *B. orientalis* under industrial setup. Besides, the different results of the parameters for the in-house authentic sample and the commercial sample are evidently indicative of the necessity of the study. Overall, this in-depth study generated the physicochemical data of the homoeopathic drug *B. orientalis* for the first time.

Acknowledgement

The authors are thankful to Prof. H. Ramakrishna and all the supporting staffs of DSU (H), CCRH, Hyderabad, Telangana, India.

Financial support and sponsorship Nil

Conflicts of interest

None declared.

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ब्लाटा ओरिएंटलिस की होम्योपैथिक औषधि का भौतिक रसायन अध्ययन

परिचयः ब्लाटा ओरिएंटलिस या ओरिएंटल कॉकरोच जिसे पानी का कीट या ब्लैक बीटल भी कहा जाता है, कीट वर्ग और ब्लाटिडे परिवार से संबंधि ात है। होम्योपैथी में, इससे तैयार औषधि का उपयोग कई रोगों में किया जाता है, खासतौर पर अस्थमा के उपचार में।

सामग्री और विधिः भौतिक–रसायन अध्ययन में विभिन्न मानकों का मूल्यांकन शामिल है जैसे बाह्य पदार्थ, नमी की मात्रा, कुल भरम, जल विलेय और अम्ल विलेय भरम निधि, अनिर्मित औषधि का जल और एथनॉल उद्धरण। भौतिक–रासायनिक अध्ययन जैसे ऑर्गेनोलेप्टिक विनिर्देशों, तलछट, विशिष्ट गुरूत्वाकर्षण निर्धारण, कुल ठोस पदार्थ, पीएच–मापन, टीएलसी (थिन लेयर क्रोमैटोग्राफी), यूवी स्पेक्ट्रा और एल्कोहल की मात्रा दोनों इन–हाउस और व्यवसायिक मदर टिंचर (तैयार उत्पादों) के लिए मानकीकृत और प्रस्तुत की गई है।

परिणामः अध्ययन इंगित करता है कि इस औषधि के प्रांरभिक मानकों के मूल्य पादप औषध के लिए प्रतिवेदित की गई श्रेणियों से काफी अलग हैं। टीएलसी अध्ययन तैयार औषधि की संरचना की जटिलता की पृष्टि करता है।

निष्कर्षः ब्लाटा ओरिएंटलिस औषधि का भौतिक–रसायन डे<mark>टा इसके</mark> व्यावसायिक रूप से उपलब्ध औषधि के प्रमाणीकरण, गुणवत्ता आश्वासन और गुणवत्ता नियंत्रण प्रक्रिया के लिए मानक के रूप में कार्य कर सकता है।

Étude physico-chimique du médicament homéop<mark>athiqu</mark>e Blatta orie<mark>n</mark>talis

RÉSUMÉ

Introduction: *Blatta orientalis* ou cafard d'orient, également connue sous le nom de water bug (insecte d'eau) ou scarabée noire, appartient à la classe insecta et à la famille des Blattidae. Le médicament Blatta orientalis est utilisé en homéopathie pour traiter plusieurs maladies, notamment pour l'asthme. **Matériels et méthodes:** L'étude physico-chimique comprend l'évaluation de différents paramètres, à savoir les matières étrangères, la teneur en humidité, la teneur totale en cendres, les valeurs de cendres solubles dans l'eau et de cendres insolubles dans l'acide, les principes actifs du médicament brut extraits à l'aide d'éthanol et d'eau. Les études physico-chimiques à savoir les spécifications organoleptiques, les sédiments, la détermination de la densité spécifique, la totalité des solides, la mesure PH, la CCM (chromatographie en couche mince), les spectres UV-Vis et la teneur en alcool ont également été normalisés et présentés pour la fabrication de teintures mères (produits finis) à l'échelle non commerciale et à l'échelle commerciale.

Résultats: L'étude indique que les valeurs des paramètres préliminaires de ce médicament sont très différentes des gammes signalées pour les médicaments d'origine végétale. L'analyse de la CCM confirme la complexité de la composition du médicament préparé. **Conclusion:** Les données physico-chimiques du médicament *Blatta orientalis* peuvent servir de normes de la pharmacopée aux fins d'authentification, d'assurance de la qualité et des processus de contrôle de la qualité du médicament commercialisé.

Estudio físico-químico del medicamento homeopático Blattaorientalis

RESUMEN

Introducción: La *Blattaorientalis* o cucaracha oriental también conocida como chinche de agua o escarabajo negropertenece a la clase de insectos y a la familia Blattidae. Los medicamentos homeopáticos preparados a partir de la *Blattaorientalis* se utilizan en una serie de patologías, especialmente en el tratamiento del asma.

Materiales y métodos: El estudio físico-químico incluye la evaluación de diferentes parámetros, a saber, materiales extraños, contenido en humedad, cenizas totales, valores de cenizas hidrosolubles e insolubles en ácido, y extractos acuosos y etanólicos del medicamento crudo. Asimismo, se han estandarizado y presentado estudios físico-químicos, a saber, especificaciones organolépticas, sedimentos, determinación de la gravedad específica, sólidos totales, medición del pH, TLC (*ThinLayerChromatography*- Cromatografía de capa fina), espectros UV-Vis y contenido en alcohol, tanto de las tinturas madre internas como de las comercializadas (*productos acabados*).

Resultados: El estudio indica que los valores de los parámetros preliminares de este medicamento son notablemente diferentes de los rangos referidos para los medicamentos vegetales. El estudio por TLC confirma la complejidad de la composición del medicamento preparado.

Conclusiones: Los datos físico-químicos del medicamento*Blattaorientalis* pueden servir como estándar farmacopéico para la autentificación, el aseguramiento y el proceso de control de calidad del medicamento comercializado.

Physikalisch-chemische Untersuchung der homöopathischen Droge von Blatta orientalis

ABSTRAKT

Einführung: Blattaorientalis oder orientalische Schabe, die auch als Wasserkäfer oder Schwarzkäfer bekannt ist, gehört zur Klasse Insecta und Familie Blattdae. In der Homöopathie wird die daraus hergestellte Medizin bei einer Reihe von Beschwerden verwendet, insbesondere bei der Behandlung von Asthma

Material und Methoden: Die physikalisch-chemischeStudiebeinhaltet die Bewertungverschiedener Parameter, nämlich: Fremdkörper, Feuchtigkeitsgehalt, Gesamtasche, wasserlösliche und säureunlöslicheAschegehalte, Wasser- und Ethanolextrakte der Rohdroge. Physikalisch-chemischeStudiennämlichorganoleptischeSpezifikationen, Sediment-, Dichte-, Gesamtfeststoff-, pH-Wert-, TLC- (Dünnschichtchromatographie), UV-Vis-Spektren und Alkoholgehaltwurdenebenfallsstandardisiert und fürsowohlhauseigenealsauchkommerzielleUrtinkturen (Endprodukte) präsentiert.

Ergebnisse: Die Studie zeigt, dass die Werte der vorläufigen Parameter dieses Medikaments sich stark von den für pflanzliche Arzneimittel angegebenen Werten unterscheiden. TLC-Studie bestätigt die Komplexität der Zusammensetzung des Präparats

Schlussfolgerung: Die physikochemischenDaten des Arzneimittels, Blattaorientalis, könnenalsArzneibuchstandardfür die Authentifizierung, Qualitätssicherung und Qualitätskontrolle des im Handel erhältlichenArzneimittelsdienen.

順勢療法藥物東方蜚蠊(蟑螂)(Blattaorientalis)的化學病理學研究

摘要

簡介:東方蜚蠊或東方蟑螂,又稱水蝽或黑甲蟲,是屬於昆蟲類和蜚蠊科。在順勢療法中,由其製備的 藥物適用於許多疾病,特別是適用於哮喘的治療。

材料與方法:本物理化學研究包括不同參數的評估,即原藥物中的雜質、水分、總灰分、水溶性灰分 值和非酸溶性灰分值、水和乙醇提取物。物理化學研究,即感官指標、沉澱物、比重測定、總固體含 量、pH測定、TLC(薄層色譜)、紫外-可見光譜和酒精含量,同時把內部和商業母酊(成品)標準化及 展現出來。

結果:研究顯示,該藥的初步參數與植物藥物所報告的範圍有很大不同。TLC研究證實了此製備藥物成分的複雜性。

結論:藥物東方蠊的物理化學數據可以作為此商業藥物的認證、質量保證和質量控製過程的藥典標準。