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Original Article

Comparative Study for Methods of Extraction of Harmala Alkaloids

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ABSTRACT

Peganum harmala L. (Zygophyllaceae) is a wild flowering plant grows in Egypt and Mediterranean region. The seeds of this plant contain 2-5% Harman alkaloids which retain diverse pharmacological activity. Therefore, developing an efficient and economic method for extraction the alkaloids will pose a great importance for both medical and economical aspects. Microwave assisted extraction (MAE) and Ultrasonic assisted extraction (UAE) were used in comparison to the commonly used Soxhlet extraction method. MAE presented the highest yield of alkaloids (4.7 and 4.8%) after 15 and 30 minutes respectively. Meanwhile UAE produced 3.7 and 4.3% after 15 and 30 minutes respectively. However conventional Soxhlet extraction yielded 4.9 % after 7 hours of complete exhaustion extraction. This study revealed that MAE proved to be the most efficient and economic method of extraction for harmala alkaloids in comparison to other commonly used methods.

Keywords: *Peganum harmala* seeds; Microwave assisted extraction; Ultrasonic assisted extraction; Soxhlet extraction.

INTRODUCTION

Peganum harmala L. (Zygophyllaceae), commonly called as Syrian rue, African rue and wild rue [1]. It is a perennial, bushy and wild-growing flowering plant with short creeping root which may grow to 30-100 cm high as shown in **Fig.1a**. The plant is widely distributed mainly in the Mediterranean region, also found in Central Asia, North Africa and also cultivated in America and Australia [2,3]. A red dye, from the seeds is often used to dye carpets and wool. The stems, roots and seeds are used to make tattoos, inks and stains. According to literature, *Peganum harmala* L., shows different pharmacological activities like antioxidant [4], anti-leishmanial [5], anti haemosporidian [6], antihistaminic [7], vasorelaxant [8], antitumor [9], wound healing [10], antiplasmodial [11], MAO inhibition [12], DNA topoisomerase 1 inhibition [13], antibacterial and anti-tubercular activity [14], myeloperoxidase inhibition [15]. It is also reported to have antifertility [16], analgesic [17], anticancer [18] and anti-nociceptive properties 19]. *P. harmala* mainly contains β-carboline alkaloids and contains up to 2 - 5% total alkaloids, some major phytoconstituents reported from it are harmine, harmaline, harmane and harmalol shown in (**Fig.2**)[20].

Conventional methods of extraction which used from long time till nowadays like maceration and Soxhlet extraction may lack selectivity, time consuming, give lower yields and present environmental risks. Also it may cause oxidation and/or hydrolysis of target extracted compounds due to long exposure to high temperature and long extraction times [21]. To overcome these drawbacks, alternative methods of extraction have been used for replacing the conventional one, for example, the use of ultrasonic assisted extraction, pressurized solvent extraction and microwave assisted extraction [22]. The use of microwave energy as a heating source in analytical laboratories started in the late 1970s and was applied to acid digestions [23]. The development of microwave assisted extractions was first reported by Ganzler and co-workers [24]. Microwaves interact selectively with the polar molecules present in glands, trichromes or vascular tissues. Localized heating leads to the expansion and rupture of cell walls and is followed by the liberation of essential gradients into the solvent [25]. Many reports on the beneficial effects of ultrasonic and microwave extraction with respect to natural products have been published, with significant improvements over conventional extraction methods offering much lowered extraction time and enhanced efficiency [26]. So this study intends to compare

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different extraction methods; Soxhlet extraction, Microwave assisted extraction and ultrasonic assisted extraction to develop the best efficient, economic and simple method for extraction of *P. harmala* alkaloids.

EXPERIMENTAL

Materials: *Peganum harmala* seeds were purchased from local market in Mansoura city, Egypt in April 2015 (**Fig.1b**). The seeds of the plant were ground into fine powder using coffee blender. All chemicals and reagents used were in analytical grade.

Methods: Kartal method was used (*Kartal, et al. 2003)* [27] with some modifications. Ten grams of dried and powdered seeds of *Peganum harmala* were extracted with 200ml of 70% methanol using different 4 methods of extraction:

a. Microwave extraction by placing the powdered seeds with the solvent in 500 ml-rounded bottom flask in CEM microwave system [MarsModel, 907500, USA] 800W, adjusting it at 50C° for 15 and 30 minutes (**Fig.3**).
b. Soxhlet extraction at temperature 50C° for 7hrs (till the complete exhaustion), 15 min and 30 min.
c. Ultrasonic extraction by using 24 kHz probe [Hielscher UP400S ultrasonic processor, 400W] for direct sonication extraction in 250ml beaker containing the plant material and the solvent [the horn tip position inside the extraction vessel was 1cm under the solvent level] showed in (**Fig.4**) at time intervals 15 and 30 min at room temperature
d. Indirect ultrasound-assisted extraction using sonication bath. The plant material and the solvent were placed in a 250 mL Erlenmeyer flask (8 cm bottom diameter attached to a condenser from the top and immersed in an ultrasonic bath (UD100SH-2.8LQ Memory Quick, 40Khz). The extraction mixture in the conical flask was kept below the water level of the bath about 4 cm from the bottom of the flask, exactly over the ultrasonic transducer. The temperature of the mixture was adjusted at 50C° for 15 and 30 minutes.

The extracts were evaporated to dryness. The residue was dissolved in 100ml 2%HCl, filtered throughout a "Fisher brand QL100, 150 mm filter paper". The filtrate was extracted twice with 50 ml petroleum ether. The aqueous acid layer was basified (pH 10) with 50ml dil. NH_4OH and then extracted 4 times with 50 ml Methylene Chloride. The methylene chloride layers were combined and evaporated to dryness, and the total alkaloids were weighed out.

Determination of total Harmala alkaloid contents: Qualitative analysis of the total alkaloids using TLC: Analytical chromatography was used to verify the presence of alkaloids at least a majority in the extract. TLC plates ready to use (silicagel 60F-Merck brand aluminum support). The mobile phase used was methylene chloride: methanol 9:1 (V/V). The extract is dissolved in 1 ml of methanol. After dissolution of the sample in methanol, we deposit10µl of the solution (extract) using a micropipette on the plate 1 cm the lower edge of the baseline. Each deposit was dried. The plate is then placed in the chamber containing the mobile phase migration, When the solvent front reaches 1cm from the top of the plate, the chromatogram plate is removed, dried and vaporized with Dragendorff reagent till the appearance of orange colored spots(**Fig.5**).

FTIR spectroscopy: Detection of the IR peaks can help in determination of function groups present in the extract which is useful in confirmation the presence of harmaline and harminealkaloids [28,29].

RESULTS AND DISCUSSION

Harmala alkaloids have been used for many decades for different pharmacological uses, so exploring the optimum method with high yield and time saving is very important. Our study resulted that there is wide variation between the four methods of extraction for fifteen and thirty minutes, only continuous extraction by Soxhlet extraction used for complete exhaustion which took seven hours.

Tab.1 and Fig.6 representing the amount of alkaloids extracted from each method in grams. The largest alkaloids yield obtained from microwave assisted extraction method which reached 4.7% and 4.8% for time intervals 15 and 30 minutes respectively. Extraction times in microwave assisted extraction are very short compared to conventional techniques (Soxhlet) and usually vary from a few minutes to a half-hour, by increasing extraction time it tends to increase the extraction yield. However, this increase was found to be very small with longer time as showed (only 0.1 % increase from 15 minutes to 30 minutes).

Fig.7 represent the percent of alkaloids extracted by the four methods for the same time of extraction (15 minutes). Microwave assisted extraction is the highest yield followed by direct sonication and indirect sonication (sonication bath), while the

Soxhlet extraction showed the lowest obtained amount of alkaloids.

In microwave assisted extraction, the process acceleration and high extraction yield may be the result of a synergistic combination of two transport phenomena: heat and mass gradients working in the same direction. On the other hand, in conventional extractions the mass transfer occurs from inside to the outside, although the heat transfer occurs from the outside to the inside of the substrate.

The relatively high yield obtained from the direct ultrasonic extraction thought to be due disruption of the cell wall of seeds by cavitation effect, which lead to bubbles in the solution which collapsed releasing high pressure and a very speed jet causing the direction the solvent particles towards the plant material giving a significant reduction in the extraction time and an increase in the yield of alkaloids [30].

Although the short time interval (15min) showed high alkaloids yield for direct sonication than the indirect one (bath), the long time (30min) showed high yield for the indirect sonication bath, and that is may due the effect of the temperature for long time in extraction process as we set the temperature of the bath at 50Cº.

Fig.5 present the TLC of the different methods using solvent system methylene chloride to methanol 9:1 and sprayed with Dragendorff reagent. Three spots are separated on the plate with two major orange spots at Rf of 0.4 and 0.7 indicating the major two alkaloids harmaline and harmine respectively. These values for both Harmine and Harmaline were in agreement with the reported values in the literature [28].

All FTIR charts possess the same peaks indicating the presence of the same alkaloids in all methods of extraction. The spectra of *P. harmala* extracts were in accordance with harmala alkaloids standards and the absorptions of *P. harmala* extract at different wave lengthValues1072, 1568, 2826, 2925 and 3423 refers to different functional groups C-N stretching vibration, aromatic C=C bending vibration, OCH_3 stretching vibration, aromatic C-H asymmetric stretching vibration and N-H stretching vibration respectively which confirms the presence of harmala alkaloids in the extracts [28,29]. All methods FTIR spectra are represented in (**Fig.8-11**).

CONCLUSION

From our results demonstrated in this paper we can clearly conclude that the microwave assisted extraction (MAE) process is the most optimum method for extraction of Harmala alkaloids from its seeds. Since MAE yielded 4.7% alkaloids only in fifteen minutes which approximately equal the amount yielded from complete exhaustion by Soxhlet extraction which took seven hours yielding 4.9%. Direct and indirect sonication assisted methods showed better alkaloids yield than the conventional method but still not as much as the MAE.

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Tab.1: Total alkaloids yield (gm) produced from different methods after different time

Extraction Method	**Time (min)**	**Total alkaloid Yield (gm)**
1. Microwave extraction	15 min 30 min	0.47 0.48
2. Soxhlet	7 hrs. 15 min 30 min	0.4904 0.316 0.430
3. Ultrasonic extraction Direct Indirect	 15 min 30 min 15 min 30 min	 0.373 0.377 0.349 0.434

Fig.1: a: *Peganum harmala* plant and its fruits [31], b: *Peganum harmala* seeds (2 X)

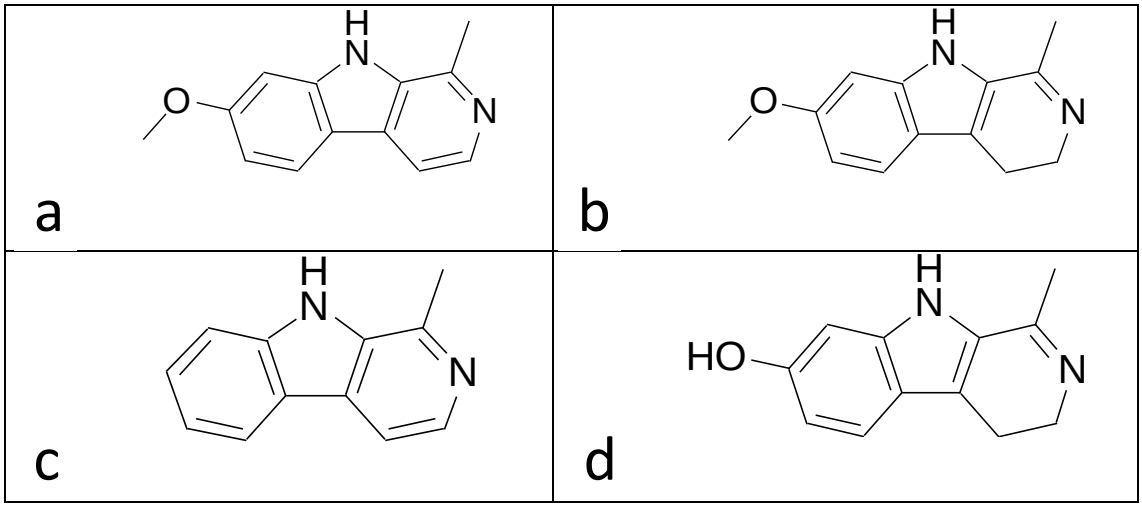

Fig.2: Chemical structure of the major Harmala alkaloids a: harmine, b: harmaline, c: harmane, d: harmalol.

Fig.3: Microwave extraction device: CEM microwave system (Mars Model, 907500, USA).

Fig.4: Direct sonication device (Hielscher UP400S ultrasonic processor, 24 kHz) a: left side view of the device b: right side view of the device c: Horn tip of the direct sonication device d: Extraction process with direct sonication placing the horn tip inside 250ml beaker containing plant material with the solvent.

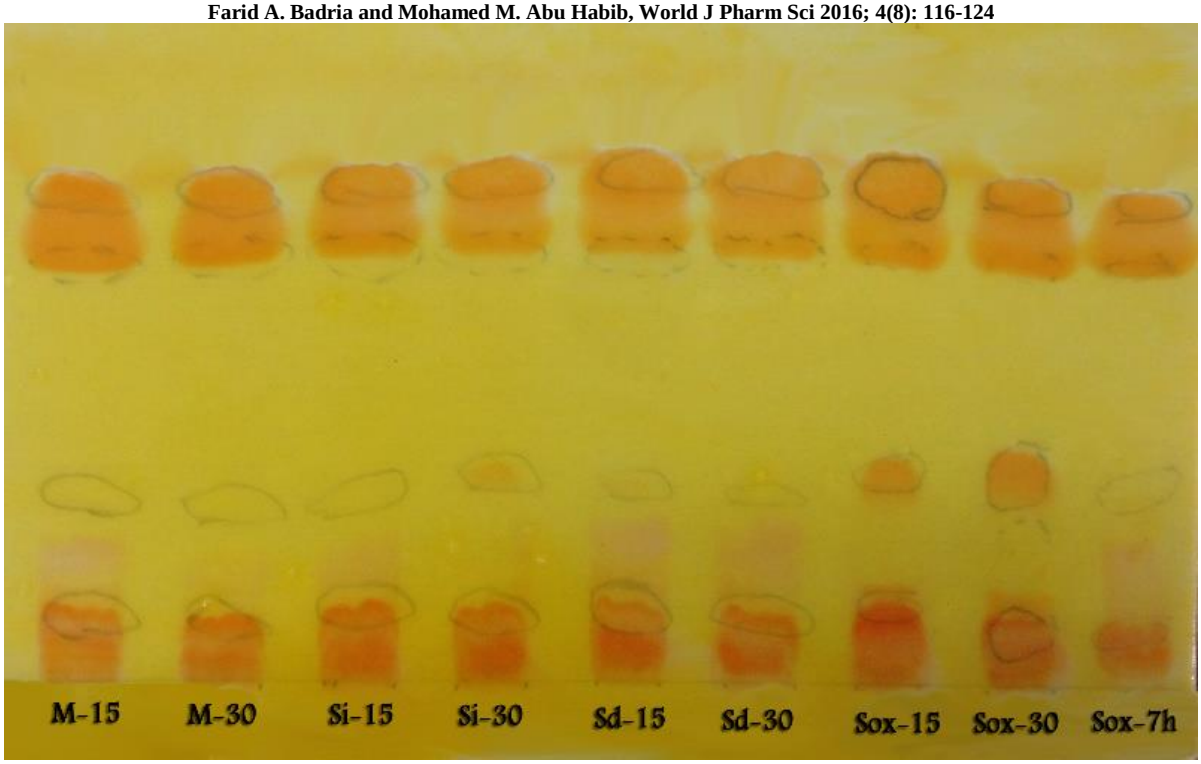

Fig.5: TLC for different extraction methods using solvent system (methylene chloride to methanol 9:1) sprayed with Dragendorff reagent M-15: microwave after 15 min, M-30: microwave after 30 min, Si-15: sonication indirect after 15 min, Si-30: sonication indirect after 30 min, SD-15: sonication direct after 15 min, SD-30: sonication direct after 30 min, Sox-15: soxhlet after 15 min, Sox-30: soxhlet after 30 min, Sox-7h: soxhlet after 7 hours.

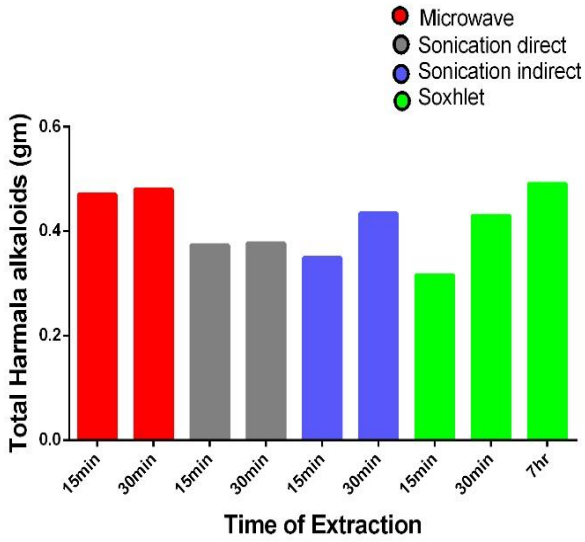

Fig.6: Amount (gm) of total alkaloids extracted using different methods at different times.

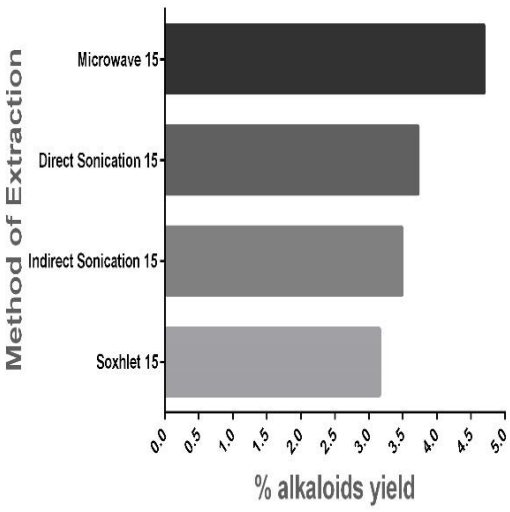

Fig.7: Percent of total alkaloids extracted using different methods after 15 min.

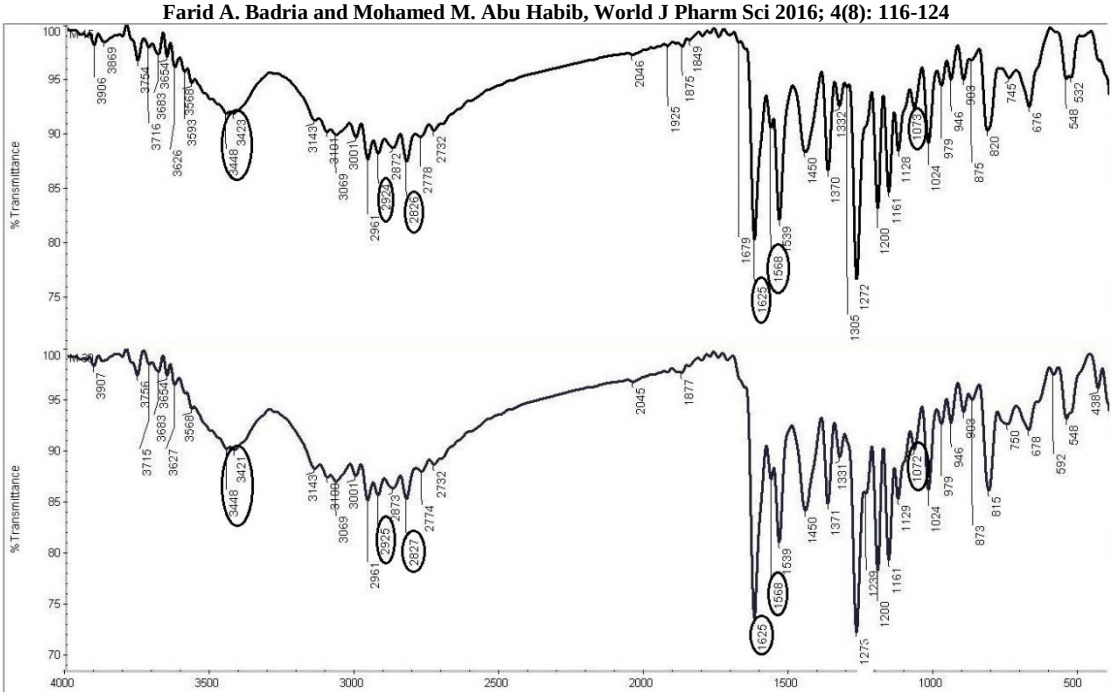

Fig.8: IR chart of alkaloid extracts by microwave assisted extraction after 15 min and 30 min respectively with major characteristic peaks of harmala alkaloids which are encircled (λ: 1072, 1568, 2826, 2925 and 3423).

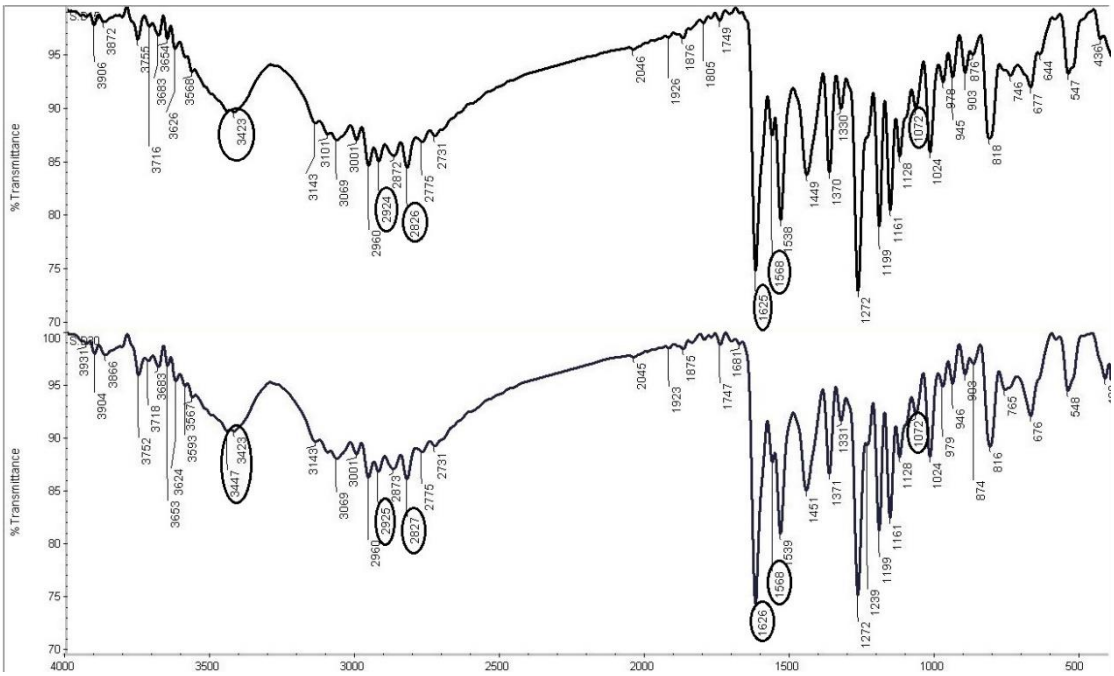

Fig.9: IR chart of alkaloid extracts by Ultrasonic assisted extraction (direct) after 15 min and 30 min respectively with major characteristic peaks of harmala alkaloids which are encircled (λ: 1072, 1568, 2826, 2925 and 3423).

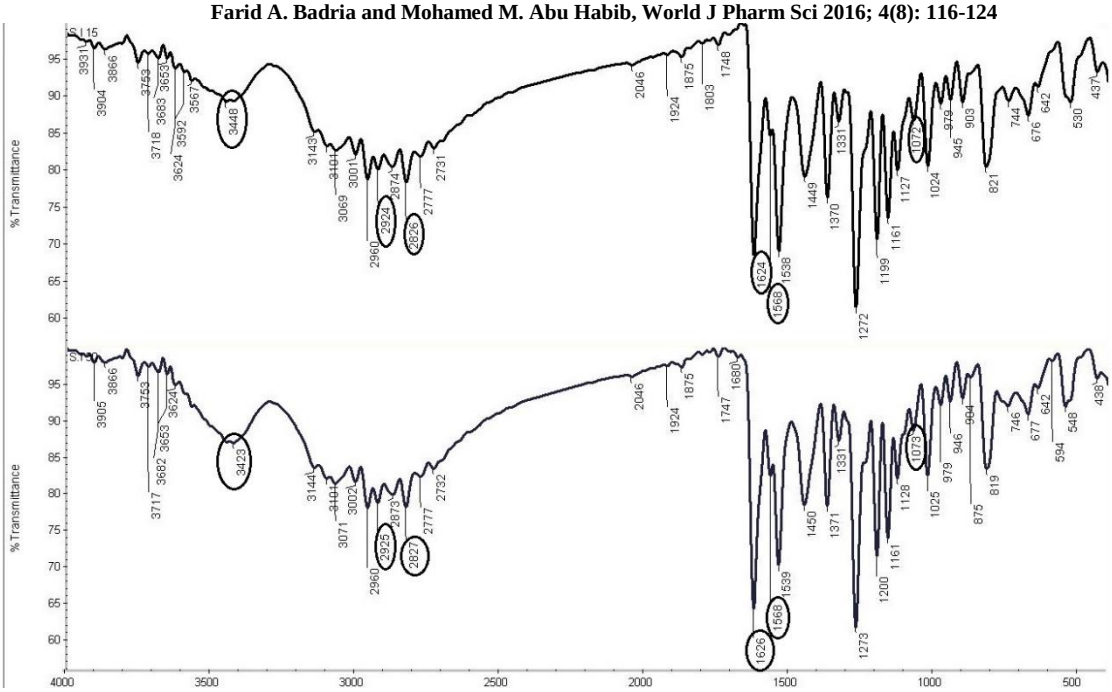

Fig.10: IR chart of alkaloid extracts by Ultrasonic assisted extraction (indirect) after 15 min and 30 min respectively with major characteristic peaks of harmala alkaloids which are encircled (λ: 1072, 1568, 2826, 2925 and 3423).

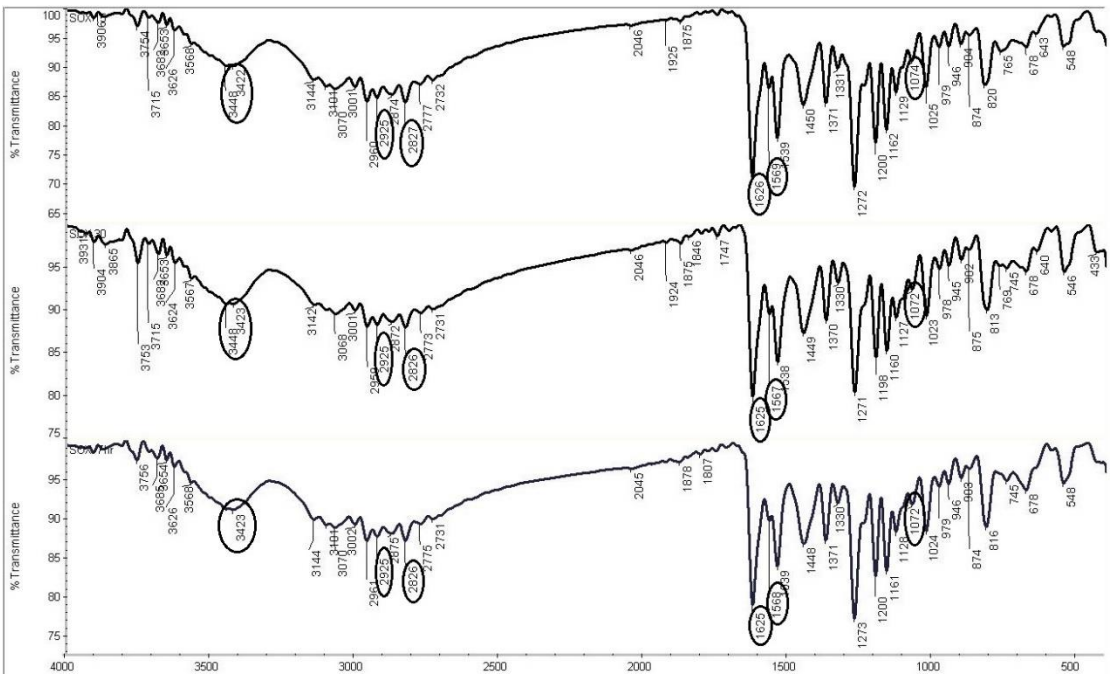

Fig.11: IR chart of alkaloid extracts by Sohxlet extraction after 15 min, 30 min and 7hrs respectively with major characteristic peaks of harmala alkaloids which are encircled (λ: 1072, 1568, 2826, 2925 and 3423).

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