

HPLC Tracing of Sennosides and Quality Control Study of Laxative Herbal Medicine “Al-Halol”

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ABSTRACT

Al-Halol is a famous herbal recipe, formed of senna and supportive herbal remedies. It is distributed widely in the herbal markets of Saudi Arabia (KSA) and other Gulf countries. Al-Halol is prescribed for intestinal evacuation and treatment of different forms of constipation. The aim of the present study was to deal with the quality control (QC) status of three different formulations of “Al-Halol” (Lax-1, Lax-2 and Lax-3). A simple method was designed for isolation of crude sennosides fraction from standard senna leaves and utilized for HPLC tracing of sennosides in the tested herbal medicines along with several qualitative and quantitative QC tests. The present study revealed tentatively the presence of 3.6 % senna pods, 29.1% senna leaves and 22.6 % senna leaves as principle material in Lax-1, Lax-2 and Lax-3 respectively, percentage of intestinal motility in mice was also determined as 28.4%, 65.19% and 60.73% for Lax-1, Lax-2 and Lax-3 respectively compared with negative control (54.78%), and positive controls (i.e. senna leave extract = 81.2 %, and bisacodyl =76.7 %). There was a statistically significant difference between all groups (p value < 0.001), except between standard senna leaves extract and bisacodyl and between Lax-2 and Lax-3 extracts. However, great variations have been recorded between the different samples. The present work provides a simple method for isolation and HPLC tracing of sennosides in senna drugs. It is the first time for evaluation and QC study of these laxative herbal formulations.

Keywords: Al-Halol; quality control; laxative herbal medicine; HPLC tracing of sennosides.

INTRODUCTION

The use of herbal medicines for treatment of diseases was documented several thousand years ago and continue to grow steadily nowadays toward the future (Ekor, 2014). The safety and efficacy of herbal medicine, as well as QC have become important concerns for both health authorities and the public (Zeng *et al.*, 2011; WHO, 1993). World health organization (WHO) has also issued guidelines for the safety monitoring and assessment of herbal medicines (WHO, 2004).

Constipation is a common functional bowel disorder that affects many people, and includes difficulty passing stool, hard stool, and feelings of incomplete evacuation (Pasanen, 2014). Traditional Arab medicines (TAM) is preferred and

used by 40–59% of the population in KSA (WHO, 2019). The use of Arabic senna (*Cassia angustifolia*) can be traced back to the beginning of the Islamic civilization in the 7th century, where it is approved and recommended by the prophet MOHAMMAD (PBUH) 14 century ago (Al-Kazweini, 1975). Arabic people usually use senna drink once or twice a year even without constipation for all ages starting from 6th year age in order to clean the gastrointestinal tract (GIT) from the reserved toxins. Arabic senna is described as laxative drugs in many traditional arabic textbooks for example (Al-Asfahani, 2006; Al-Antaky, 1980). The word “Al-Halol” is famous Arabic name for senna-containing herbal medicines. Due to intolerable side effects of senna drugs (e.g. flatulence, cramps, gripping, and GIT

problems), the herbal practitioners intentionally add some volatile oil-containing drugs like fennel, anise, cumin and caraway among others to overcome these side effects. Many people however mistakenly believe that herbal products are always safe. Although most herbal medicines are relatively safe in comparison with modern synthetic drugs, results from toxicological studies show adverse reactions to some herbal drugs which in some cases might show severe or lethal effects (Lüde *et al.*, 2016). Laxative herbal medicines, like other multi-formulated herbal products, are not exception. Unfortunately, the majority of herbal medicine are distributed in herbal markets without quality assessment (van den Berg *et al.*, 2011; Zeng *et al.*, 2011), this opens the door for very annoying and dangerous safety concerns. van den Berg *et al.*, 2011 mentioned many reports of case studies worldwide that deal with toxicities related to adulterations, admixing, or improper storage of herbal material as well as the side effects of the original herbal drugs (Izzo *et al.* 2016). The present study deals with QC tests of herbal medicine. These QC tests include botanical, chemical, and pharmacological characterization of this formula "Al-Halol" from three different herbal markets. The present study is very useful for the community including herbalists, herbal consumers, as well as Government authorities especially the health care providers.

MATERIAL AND METHODS

Herbal medicines

Three samples of the herbal formulation "Al-Halol" were purchased from three different herbal medicine shops: (Lax-1) from Hafer Elbatan city (Eastern region, KSA), while (Lax-2 and Lax-3) were purchased from Rafha city (Northern Border region, KSA). In addition to standard senna leaves (*Cassia angustifolia*), senna pods (*Cassia angustifolia*), were purchased from herbal medicine shop at Rafha city, KSA. These herbal materials were identified and voucher specimens are deposited in the department of phytochemistry and natural products. Northern Boreder University.

Apparatus, Chemicals and reagents

HPLC apparatus [Waters® 2545 Quaternary Gradient Module pump and equipped with Waters® 2998 diode array detector using Empower™ 3 Software], light microscope (Micros-Austria), spectrophotometer (APEL-Japan), rotary evaporator (heidolph - Germany), vortex (VELP Scientifica - Italy), pH meter (Mettler Toledo

- Switzerland), centrifuge (Hettich - Germany), and sensitive balance (A&D - japan), were used in the experimental study. All solvents, reagents and standards were purchased from Sigma-Aldrich Chemical (St. Louis, MO, USA) and Merck (Darmstadt, Germany).

Macroscopic and microscopic examination

Macroscopic characterizations (organoleptic tests, including condition, appearance, color, odor, taste) and microscopic characterizations (microscopical examination of the fine powdered herbal formulae under light microscope for detection of the main diagnostic micro-botanical elements e.g. types of epidermal cells, trichomes, sclerides, fibers, parenchyma cell and xylem vessels) of herbal materials were done according to standard procedure (WHO, 2011) after mounting in distilled water or glycerinated distilled water and different characteristic dyes, like safranin, iodine, phloroglucinol/HCl, reuthenium red, methylene blue, sudann III and FeCl₃.

Quantitative QC tests

Quantitative QC tests including loss on drying, Determination of total ash, acid-insoluble ash, Water-soluble ash and Determination of extractive matter using 70% methanol extraction and water extraction were carried out according to standard procedure (WHO, 2011). Two grams finely powdered herbal material in a previously ignited crucible was ignited in an oven at high temperature (500-600 °C) until it white (free from carbonated residues). The net weight (wt) of the carbon-free ash was determined and the percentage (%) of total ash was calculated. Known amount of the total ash was dissolved in 2N HCl solution in a suitable dry previously ignited crucible was boiled for 5 min. The total insoluble ash was collected on ashless filter paper and ignited to obtain the % of acid insoluble ash. The % of water-soluble ash was obtained after boiling the known amount of total ash with 25 water in a suitable crucible, then the residue was collected as before and ignited. The amount of water soluble ash = total ash - water insoluble ash.

Amount of loss on drying which equal to the amount of moisture contents and volatile constituents was calculated after dry of known amount (5gm) of herbal material in an oven at 100-105 °C until constant wt., the difference in the weight of herbal powder before and after drying equals the amount of loss on drying. The amount

of extractive matter was obtained after extraction of known amount (2 g) of the finely powdered herb in 100 mL of both 70 % methanol and cold water separately in two stoppered conical flask using magnetic stirrer for 6 hours, stand for 18 hours, then 25 mL filtrate were transferred into clean dry and flat-bottomed dish. The dish was then dried carefully and completely to dryness (constant wt.) and the amount of dry extractable matter was calculated for each solvent individually. The percentage of each herbal powder was then calculated. All experiments were repeated three times and the obtained results were averaged.

Phytochemical screening

The presence or absence of different phytochemical constituents in hydroalcoholic extract of the herbal formula was tested according to the standard procedure (Shah & Seth, 2010). The standard Molisch test was applied for detection of carbohydrates and /or glycosides, where the presence of violet ring indicates the positive results. The Dragendorff's spray reagent was used for detection of true alkaloid where appearance of orange red spot on the paper spotted with a drop of the herbal extract is a positive result. Cardiac glycosides with tested with standard Baljet test (gives yellowish orange) for lactone ring containing aglycones, Keller-Kiliani test (reddish-brown to bluish-green) for deoxy-sugar parts, and Liebermann-Burchard test (violet to blue at the junction of two phases) for steroidal nucleus, positive results of the three tests are indicative for the presence of cardiac glycosides. Liebermann-Burchard test was applied as a standard test for presence of phytosterols and/or triterpenes. Tannins and or phenolic compounds were indicated with FeCl_3 test (green color with condensed tannins, and blue color for hydrolysable tannins). The presence of saponins were tested by Blood hemolysis test or by the formation of persistent froth upon shaking of the aqueous solution of the herbal preparation. The occurrence of anthraquinones (pinkish red in the alkaline phase) were detected by Borntrager's test. The presence of flavonoids (dark yellow color) was detected after addition of alkaline reagents like (NaOH, KOH, or NH_4OH).

Preparation of the samples for HPLC analyses

One gram of each sample (Lax-1, Lax-2 and Lax-3, senna leaves and senna pods) was extracted separately with 3x15 mL hydro methanol 70 % followed by 2x15mL hot distilled water (dist.

water, or dist. H_2O). The combined extract for each sample was concentrated using rotary evaporator. The final concentrated extract was adjusted to 50 mL each using dist. H_2O /HPLC-methanol (30:70) to be stock solutions of each sample. 10 mL of each stock solution were centrifuged, then 20 μL of each supernatant were used for HPLC analysis.

Isolation of purified sennoside fraction from Senna leaves

Isolation of purified sennoside fraction from Senna leaves to facilitate detection of sennoside compounds in the herbal mixtures (Lax-1, Lax-2 and Lax-3). 30 g Powdered senna leaves were extracted with 50% aqueous methanol solution till exhaustion. The total extract was then evaporated to dryness where 3.6 g dry extract were obtained. The total dry senna leaves extract was suspended in 10 mL distilled water, then extracted 5 times with benzene portions (15 mL each). The residue was kept in 250 mL Erlenmeyer flask. 100 mL cold absolute ethanol were added to the residue where brown turbidity (sennosides mixture) was obtained. The flasks were kept in refrigerator overnight, then the ethanol extract was filtered off and brown precipitate was collected, washed again with cold absolute ethanol and air dried where 1.07 g crude sennosides were obtained.

HPLC-analyses

HPLC analyses of herbal medicines (Lax-1, Lax-2 and Lax-3) were conducted in comparison with senna leaves, senna pod extracts and purified sennoside fraction from Senna leaves to facilitate tracing of sennosides (the main active compounds) in the herbal medicines. Chromatographic analysis was carried out on a 10 mm x150mm, 5 μm , 100 \AA RP-18 column. The mobile phase consisted of two solvents: HPLC-methanol (Eluent A) and water:formic acid, 100:0.2 (Eluent B). The flow rate was 4mL/min. The mobile phase gradient elution for each run was processed as follows: 0 min A/B (10:90), 5 min A/B (10:90), 17 min A/B (100:0), 24 min A/B (100: 0), 28 min A/B (10:90), 30 min A/B (10:90). The chromatograms were recorded at different wave lengths (235 nm, 254nm, 280nm and 340nm). The area under the peak method was applied for determination of amount of sennosides tentatively in each sample. These determinations based mainly on detection and calculation of area under the peak of the most obvious sennoside peak (sennoside B) at retention time = 13.0 minute for all samples of herbal mixtures, senna leaves and senna pods samples.

Oxidative hydrolysis of pure sennoside fraction

Zero point one grams pure sennoside fraction was dissolved in 10 mL distilled water, then 1mL concentrated sulfuric acid (conc H₂SO₄) was added and the reaction mixture was boiled for 5 min, then 2 mL FeCl₃ reagent were added and the reaction mixture was boiled again for additional 5 min, then cooled to room temperature and extracted with benzene to obtain the oxidative hydrolytic products of sennosides.

TLC and HPLC analysis of oxidative hydrolytic products of sennosides

TLC and HPLC analyses of the oxidative hydrolytic products were carried out to examine whether sennoside fraction is pure (hydrolytic products should contain rhein and aloemodin only) or not (the fraction shows additional natural compounds). The first prediction will prove the usefulness of this simple method for isolation and purification of sennoside fraction away from other natural compounds in senna drugs. It supports also utilization of sennoside fraction as a guide to characterization of sennoside compounds in the herbal mixture.

The oxidative hydrolytic products of sennosides were obtained after evaporation of benzene and then analyzed using TLC [stationary phase: precoated silica gel, mobile phase: n.hexane: EtOAc:MeOH; 5:4:1], and HPLC using the above mentioned gradient elution system.

Determination of sugar content

The method described by (Nielsen, 2017) with slight modifications was applied, using serial dilutions of standard sucrose solution to obtain the standard calibration curve. 50 mL sucrose stock solution in dist. water was freshly prepared (100µg/ mL); from which standard sample dilutions containing 0.0, 5, 10, 15, 20, 25, 35, and 40 µg/2mL dist. water were prepared. Test samples were prepared from the herbal medicine by 1g powder maceration in 50 mL hot distilled water for 5 min and filtered. The plant material was washed again with additional 2x 50 mL hot water and filtered. The filter paper was rinsed 2 times with additional 50 mL each. All filtrates and washing were combined, and the final volume was adjusted to 1000 mL with dist. H₂O. 0.1mL of each herbal medicine sample was added to 1.9 mL dist H₂O and treated the same as standard sucrose solutions. 0.05 mL 80 % phenol was added to each sample tube containing a total volume of 2 mL, then, Mixed on a vortex test tube mixer. 3 mL conc H₂SO₄ were

then added and mixed thoroughly on vortex. The tubes left aside for 10 min and then cooled to room temperature using water bath. The test tubes were vortexed again before reading the absorbance. The spectrophotometer was zeroed with the standard sample that contains 0.0µg sucrose (i.e., blank). The absorbance of all other samples was measured at 490 nm. The absorbance values of the standard samples were used to obtain the standard calibration curve and the equation. The equation was used to obtain the sugar concentration of the test samples.

Intestinal motility test

The effect of the extracts on intestinal motility was tested according to (Guarize *et al*, 2012). Six groups of six mice each were selected. Group 1 received vehicle (1% gum Arabic solution), Group 2 received senna leaves extract (200mg/kg), Group 3 received commercial laxative drug "bisacodyl" (0.25 mg/kg), Group 4, 5 and 6 were administered 300mg/kg of the herbal medicine extracts Lax-1, Lax-2 and Lax -3 respectively through the feeding cannula. 40 min later, 0.5 mL of 5% charcoal suspension (in 1% gum Arabic solution) was given orally for each animal under experiment. 20 min later, the animals were sacrificed by cervical dislocation and the percentage of intestinal transit of charcoal meal was calculated for each animal according to the following equation

$$\% \text{ of intestinal transit} = \frac{m \times 100}{T},$$

Where: m = the distance (in millimeters) that was travelled by the charcoal meal in the region between pylorus to caecum,

T = the total distance between pylorus and caecum.

Statistical analysis

Using SPSS program version 21 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.) the mean and standard deviation (SD) of all studied groups for standard motility test were compared using ANOVA test and post hoc test was used to identify statistical differences in-between groups.

RESULTS AND DISCUSSION

The present work gives full picture of the quality assessment of three laxative herbal medicines distributed widely in KSA herbal markets under the trade name "Al-Halol".

It also provides a simple method for isolation and HPLC tracing of dianthrone derivatives (Sennosides). However, the experiments show great variations between the three laxative herbal samples based on macroscopical and microscopical examinations, HPLC analyses, effect on intestinal motility, and other quality control tests.

Macroscopic examination

Visual inspection and organoleptic characterization should that only Lax-2 has defined herbal compositions, licensed company information, dose and method of administration, while other formulas (Lax-1 and Lax-3) are lacking of these important information. Lax-2 was purchased as 175 g entire herbal mixture formed of senna leaves (50g), rose flower (2 g), Tamarind fruit cake (20 g), *Terminalia chebula* fruits (9.5 g), *Ziziphus jujube seeds* (7.2 g), sugar lump (17.2 g), in addition to fenugreek seeds (10 g), fennel (10 g), anise (10 g), cumin (10 g) and coriander (10 g). Lax-2 has mixed agreeable aromatic odor characteristic for foenugreek seeds, fennel, anise, cumin and coriander. It has sweet, slightly acrid, mucilaginous and agreeable aromatic taste characteristic for foenugreek seeds, fennel, anise, cumin and coriander. Lax-2 was produced by Medina factory for herbal medicines in Medina city, KSA. As recommended by the factory, the total amount of Lax-2 should be immersed in 1L hot water for 2 Hours and then filtered. The filtrate is enough for two persons and taken at bed time. Lax-1 and Lax-3 were purchased as mixed powdered material, has similar agreeable aromatic odor and taste but stronger than that of Lax-2.

Microscopic examination

Microscopic examination revealed the presence of palisade-like epidermal cells (characteristic for fenugreek seeds), basket-like hypodermal cells (fenugreek seeds), branched vittae (anise fruits), unicellular curved conical warty hair (anise fruits), cross layers of thin sclerenchymatus mesocarp cells (coriander fruits), lignified reticulate parenchyma cells of mesocarp layer (fennel fruits), multicellular multiseriate non-glandular trichomes (cumin fruits), crystal sheath of calcium oxalate (senna leaves/pods), unicellular warty non-glandular trichomes (senna leaves and pods) in all three herbal samples (Lax-1, Lax-2, and Lax-3), while fragments of stellate hairs (characteristic for boldo leaves) are found only in Lax-1 sample.

Phytochemical screening

Phytochemical screening revealed the presence of carbohydrates and/ or glycosides, alkaloids, flavonoids, tannins, sterols and/ or triterpenes, and saponins in all samples (Lax-1, Lax-2 and Lax-3). Anthraquinone derivatives were relatively high in Lax-2 and Lax-3 samples compared with Lax-1, while cardiac glycosides are absent in all samples.

Results of quantitative QC tests

Great variations were found between the different formulations of "Al-Halol" through quantitative QC tests (amount of sugar contents, amount of alcohol extractive, aqueous extractive, Moisture contents (loss on drying), amount of total ash, acid insoluble ash and water soluble ash). However, the results of three experiments of each parameter, average and SD (Figure 1). It was found that the sugar content in Lax -1 (44.69 ± 0.771 %) and Lax-3 (41.5 ± 0.511 %) respectively is much higher than that of Lax-2 (15.21 ± 0.188 %). Accordingly, the percentage of both 70% methanol and water extractives were higher for Lax -1 (67.4 ± 2.49 % and 58 ± 3 %) and Lax-3 (55.1 ± 0.67 % and 44.4 ± 0.93 %) respectively compared with Lax-2 (41 ± 2.07 % and 38.84 ± 2.24 %). Controversially, the amount of moisture content (loss on drying test) in lax -2 was higher (6.16 ± 0.05 %) compared with other samples, this is obviously attributed to the increased amount of hygroscopic mucilage in Lax-2 sample which contain tamarind fruit cake.

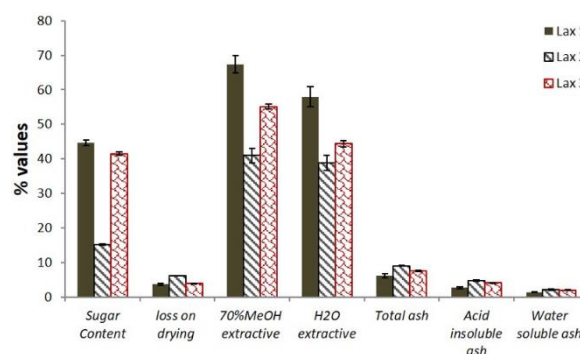


Figure 1. Quantitative QC tests results

The effect on intestinal motility

The effects of different extracts on intestinal motility were investigated. The ANOVA test was used for comparison of groups (Figure 2). Through the post hoc test, there was a statistically significant difference between all groups (p value < 0.001),

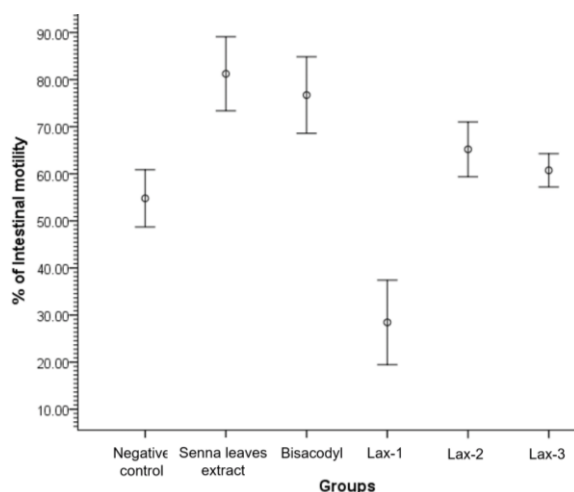


Figure 2. The effect on intestinal motility.

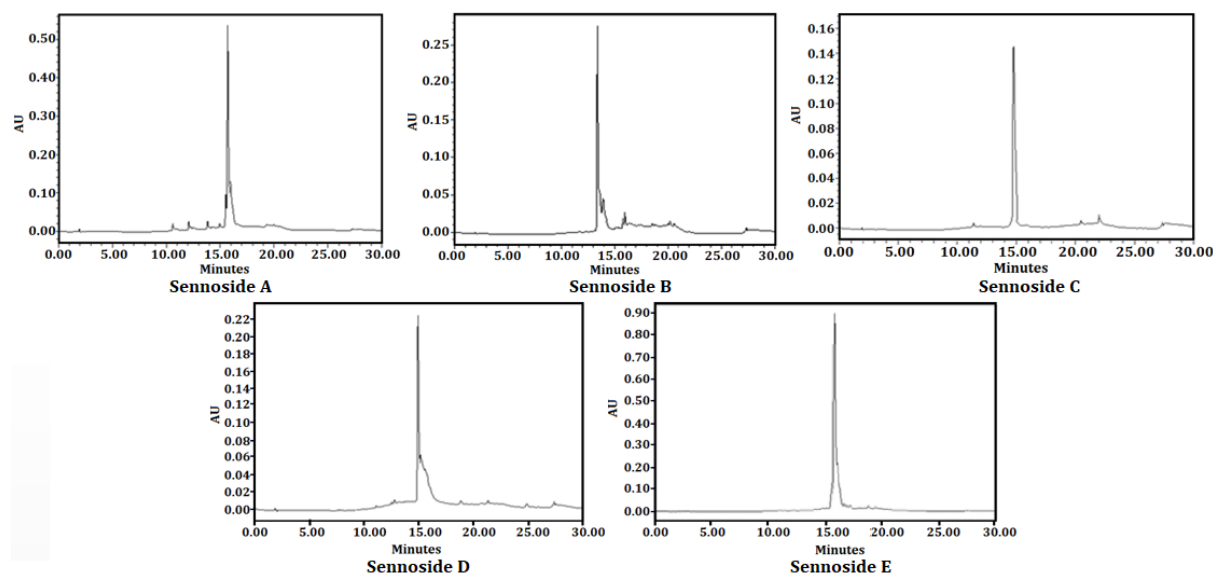


Figure 3. HPLC chromatograms of all samples: A, Lax-1; B, Lax-2 ; C, Lax-3; D. Senna leaves; E, Senna pods; F, Sennoside fraction.

except between senna leaves extract and bisacodyl and between Lax-2 and Lax-3 extracts (p value > 0.05). Lax-2 and Lax-3 extracts caused a significant increase of intestinal motility ($65.19 \pm 5.5\%$, $60.73 \pm 3.4\%$ respectively) compared with the negative control sample ($54.8 \pm 5.8\%$), while lax-1 caused a significant decrease of intestinal motility ($28.4 \pm 8.5\%$) due to the relative decrease in the anthraquinone contents and increased amount of the volatile constituents (Figure 2). Standard senna leaves extract shows the strongest effect ($81.23 \pm 7.5\%$) followed by bisacodyl ($76.72 \pm 7.75\%$).

There was a statistically significant difference between all groups (p value < 0.001), except between senna leaves extract and bisacodyl and between Lax-2 and Lax-3 extracts (p value > 0.05). Lax-2 and Lax-3 extracts caused a significant increase of intestinal motility ($65.19 \pm 5.5\%$, $60.73 \pm 3.4\%$ respectively) compared with the negative control sample ($54.8 \pm 5.8\%$), while lax-1 caused a significant decrease of intestinal motility ($28.4 \pm 8.5\%$). Standard senna leaves extract shows the strongest effect ($81.23 \pm 7.5\%$) followed by bisacodyl ($76.72 \pm 7.75\%$).

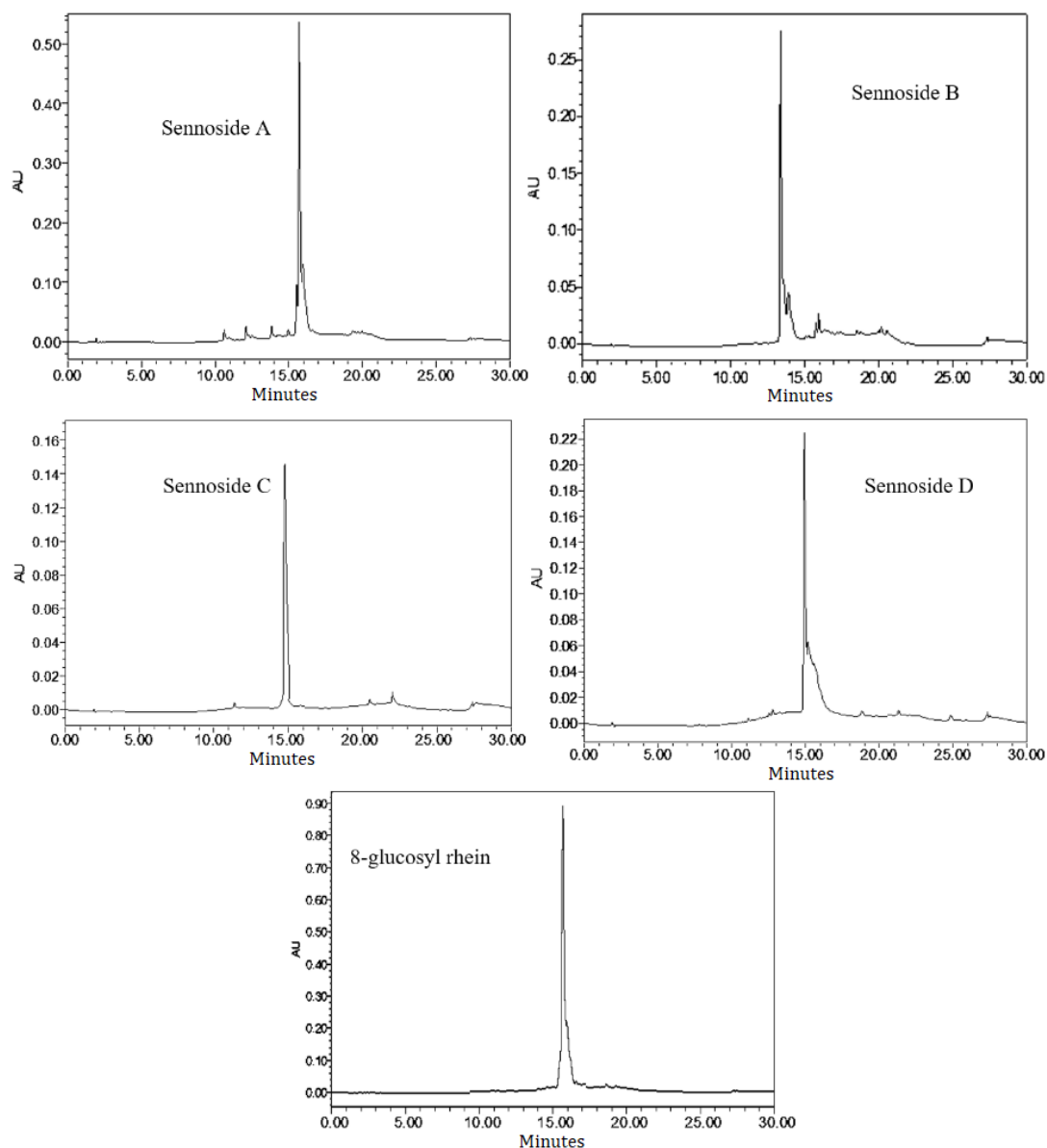


Figure 4. HPLC Chromatogram of the authentic sennoside samples.

HPLC analyses and sennosides tracing in the herbal medicine samples

HPLC chromatograms that obtained from HPLC analyses of different samples (lax-1, Lax-2, and Lax-3) in comparison with senna leaves and senna pods extracts in addition to the purified fraction of sennosides (Figure 3) and HPLC chromatograms of the authentic sennoside samples (Figure 4).

The pure sennoside fraction was prepared and utilized for tracing of sennoside peaks among

the present natural products in each sample. A magnified part of pure sennoside fraction HPLC chromatograms (Figure 5) together with the corresponding UV spectrum of each component. TLC and HPLC chromatograms (Figure 6) of oxidative hydrolytic products of purified sennoside fraction indicating two monomers only of anthracene derivatives (i.e. rhein and aloemodin) this confirms the validity of the isolation method and relative abundance of the sennosides among all test and standard samples (Figure 3).

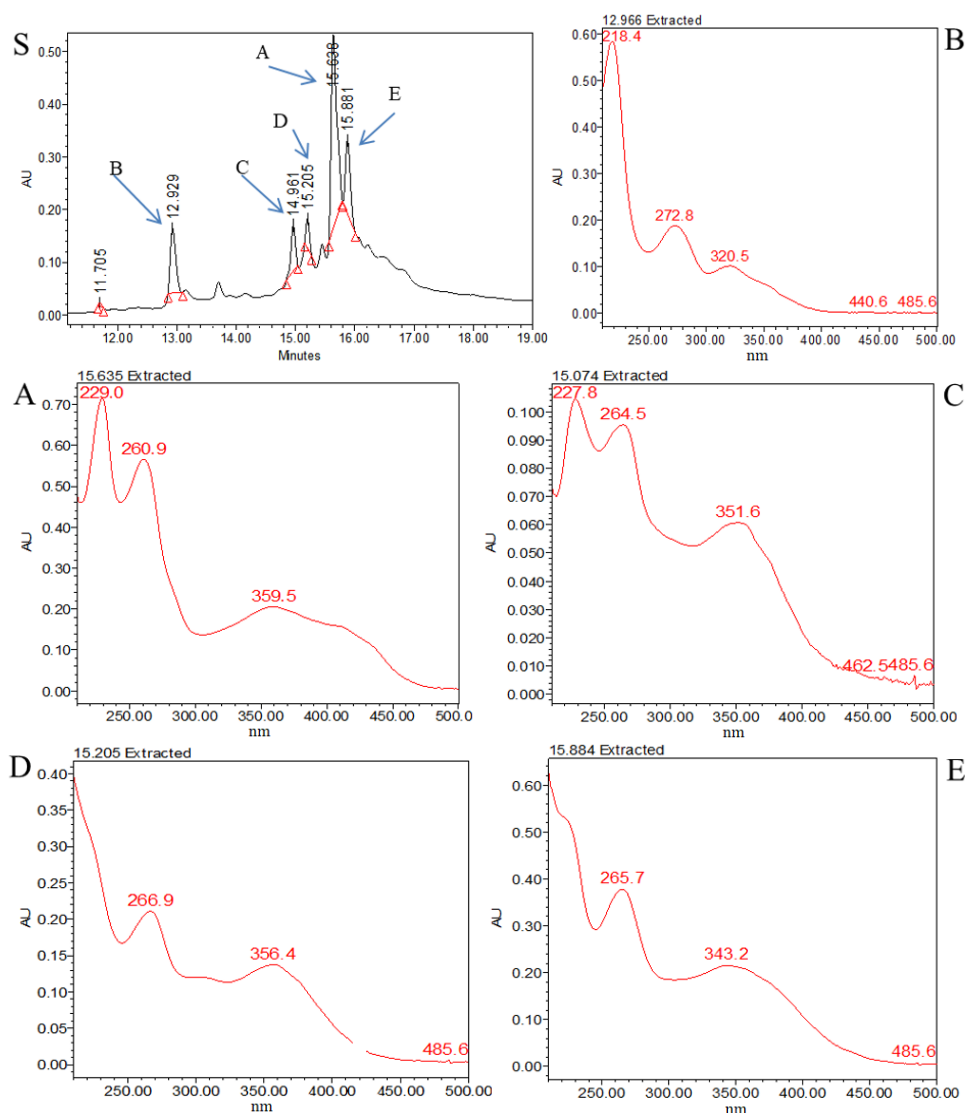


Figure 5. Magnified part of HPLC chromatogram of pure sennoside fraction and corresponding UV spectra S, magnified part of HPLC chromatogram of sennoside fraction showing corresponding peaks of sennosides A, B, C, D and 8-glucosyl rhein (E); A, B, C, D and E, are UV spectra of sennosides A, B,C,D and 8-glucosyl rhein respectively

From HPLC data and comparison of area under the peak values of the most isolated and measurable sennoside peak (sennoside B at retention time “13 minute”) we have obtained tentatively the type and quantity of senna drug in each herbal medicine. According to HPLC analyses of sennoside pure fraction, sennosides A, B, C, D as well as 8-glucosyl rhein were traced in the region from 12.0 -16.5 min on the HPLC chromatogram of each sample. The distribution pattern of the peaks related to sennosides in Lax -1 is consistent with that of senna pods, while those of Lax-2 and Lax-3 are more

similar to senna leaves extract, while the percentages of senna drug (leaves /pods), were 3.6 %, 29.1% and 22.6 % for Lax-1, Lax-2 and Lax-3 respectively.

A, HPLC chromatogram of sennoside hydrolysate; B, TLC - chromatogram (S, sennoside fraction; H, hydrolysate fraction; A and R are authentic samples of aloemodin and Rhein respectively visualize d by amonia vapour); C, HPLC chromatogram of rhein; C1, UV spectrum of rhein; D, HPLC chromatogram of aloemodin; D1, UV spectrum of aloemodin.

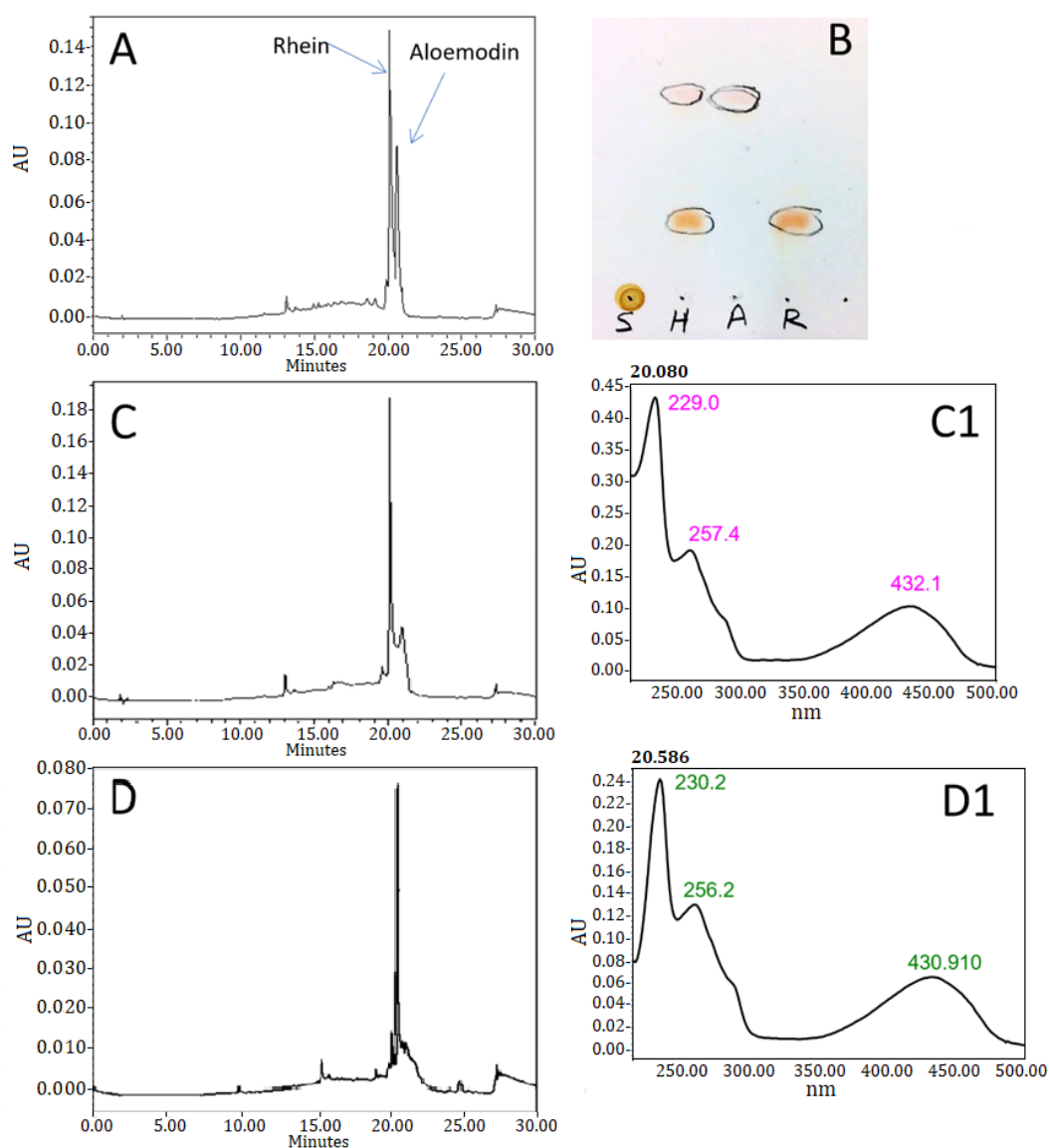


Figure 6. HPLC and TLC chromatograms of oxidative hydrolytic products of purified sennoside fraction in addition to an authentic aglycone monomers.

A, HPLC chromatogram of sennoside hydrolysate; B, TLC – chromatogram (S, sennoside fraction; H, hydrolysate fraction; A and R are authentic samples of aloemodin and Rhein respectively visualised by ammonia vapour); C, HPLC chromatogram of rhein; C1, UV spectrum of rhein; D, HPLC chromatogram of aloemodin; D1, UV spectrum of aloemodin

The obtained results were clearly demonstrated that the amount of senna drugs (or sennoside derivatives) are not optimized in all three samples and away from the pharmacopeial standard. According to (Sweetman, 2009; PDR Staff, 2000) the adult daily dose of senna leaves is 0.5 to 2 g, or 12 to 60 mg of sennosides as a single dose at bedtime. The recommended daily dose is

8.8 to 26.4 mg sennoside in children aged 6 to 12 years and 4.4 to 13.2 mg in children aged 2 to 6 years. While WHO monograph 1 (WHO, 1999) mentioned the therapeutic dose is 1–2 g of senna leaves/fruits or 10–30 mg sennosides daily at bedtime for Adults and children over 10 years. Although the acute toxicity expressed as LD_{50} of senna extract greater than 2.5 g/kg, however the

chronic toxicity can't be ignored. Toxicity of senna includes reduced body weight gain, electrolyte imbalance, liver toxicity, pruritic erythema, hepatitis, epithelial hyperplasia of the large intestine (colon and cecum) in mice. A reported case studies of Senna drugs revealed the incidence of hepatitis, IgE-mediated asthma and rhinoconjunctivitis, pruritic erythema, increased risk of colon cancer (Marvola *et al.*, 1981 & NTP report, 2012),

Also, the addition of all apiaceous fruits (Anise, Fennel, Cumin and Corianderis exaggerated. These fruits in addition to Fenugreek, *Terminalia chebula*, *Ziziphus jujuba* and Tamarind are used mainly for treatment of GIT disturbances e.g. bloating, abdominal colic and inflammation. However, these herbal medicines have some reported side effects including allergy, anaphylactic reaction, hypoglycemia, and abortifacient effects. (Gardner & McGuffin 2013; WHO, 2007; WHO, 2009; Bhadoriya *et al.*, 2011) Finally, there is an important question to be addressed; is it necessary to add all these additives or one of them may be enough? We think that no need for most of these herbal additives. It is better to modify the flavor of senna drug and minimize the side effects by addition of only one or two additional herbs in order to minimize the additional side effects of these additives and help to effectively manage and determine precisely the responsibility related to adverse reactions of this herbal formulation.

CONCLUSION

Traditional herbal medicine is an integral part of healthcare system worldwide. Senna based herbal medicine (Al-Halol) is widely distributed in the KSA herbal market. The present comparative research study revealed a quit difference between three formulations of the same herbal medicine. These differences include qualitative and quantitative parameters. The health authorities must play their specific leadership and oversight roles to control the medicinal herbal market, and to overcome the irresponsible practices through a standardized procedure for audit, handling and prescription of the herbal medicines. It is also recommended that those unqualified herbal practitioners have to join a suitable education program to be qualified and aware with these issues regarding herbal medicine practice.

Ethical approval

The experimental protocol of this work was approved by the Institutional Animal Ethics

Committee under supervision of Scientific Research Review Committee, Faculty of Pharmacy, Northern Border University, Certificate No. (PHARM-NBU-2019-2) and was performed in accordance with the guideline of the National Institutes of Health Principles of Laboratory Animal Care (NIH publication no. 85-23, revised 1985).

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