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ORIGINAL ARTICLE

Optimization of Infrared-assisted Extraction of Bioactive Lactones from *Saussurea lappa* L. and their Effects against Gestational Diabetes

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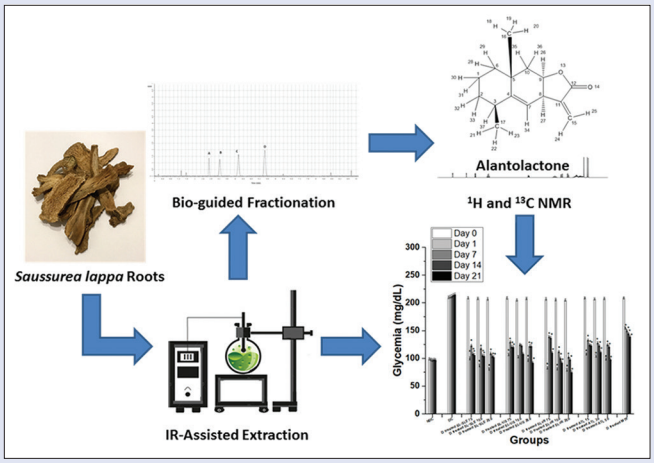
ABSTRACT

Background: *Saussurea lappa* (*S. lappa*, *Asteraceae*) have immunomodulatory effects and used in the management of many metabolic disorders. Gestational diabetes is one of the metabolic disorders affecting globally one in seven pregnant women. **Objectives:** The aim of the current study is to optimize an infrared-assisted extraction (IR-AE) method for *S. lappa* bioactive constituents, phytochemically investigate its content, isolate its most active constituent, and to assess their biological effects against gestational diabetes. **Materials and Methods:** To optimize IR-AE conditions, four main factors were studied including solvent concentration, extraction time, powder size, and IR power in the yielded extract (SL-IR). Reversed-phase high-performance liquid chromatography coupled with bio-guided fractionation and isolation procedures using ^{1}H and ^{13}C NMR method were utilized. Solid–liquid (SL-SLE) and ultrasound (SL-US) extraction methods were also done. **Results:** The optimal IR-AE extraction conditions were found to be 20% aqueous phase concentration, 60-min extraction time, 70 mesh powder size, and 70 W IR power. Phytochemically, four major lactones were identified, including costunolide, dehydrocostuslactone, isoalantolactone, and alantolactone (ATL). ATL was the most active lactone. SL-IR, SL-US, SL-SLE, or ATL showed a significant ($P < 0.05$) and dose-dependent hypoglycemia in pregnant diabetic group, adequate fetus weight percentage elevation and did not show any external anomalies. The best control of gestational diabetes, insulin secretagogue potentials, elevation in serum catalase and reduced glutathione levels, and lipid peroxidation decrease were demonstrated by SL-IR 250 mg/Kg. The antioxidant and the insulin secretagogue activities might be among the main mechanisms, whereby the SL-IR controls gestational diabetes and decreases offspring anomalies. **Conclusion:** Currently, it is the first time to optimize an IR-AE method for extracting bioactive lactones from *S. lappa*. The optimized IR-AE technique has shown to be a rapid and efficient extraction method with SL-IR showing superiority in controlling gestational diabetes for pregnant groups coupled with high safety profile on the offspring.

Key words: Gestational diabetes, infrared-assisted extraction, lactones, pharmacognosy, *Saussurea lappa*

SUMMARY

- Infrared-assisted extraction (IR-AE) method has shown to be an effective and time-conserving novel extraction method. It is the first time to optimize an IR-AE method for extracting bioactive lactones from *Saussurea lappa* (SL). SL ultrasound extract showing superiority in controlling gestational diabetes for pregnant groups coupled with high safety profile on the offspring. The best control of gestational diabetes, insulin secretagogue potentials, elevation in serum catalase and reduced glutathione levels, and lipid peroxidation decrease were demonstrated by SL infrared extract 250 mg/Kg.



Abbreviations used: IR-AE: Infrared-assisted extraction; SL: *Saussurea lappa, S. lappa*; SL-IR: *Saussurea lappa* infrared extract; SL-US: *Saussurea lappa* ultrasound extract; SL-SLE: *Saussurea lappa* solid–liquid extract; ATL: Alantolactone; NDC: Nondiabetic control; DC: Diabetic control; MTF: Metformin; TBARS: Thiobarbituric acid; GSH: Reduced glutathione; CAT: Catalase; APA: Adequate for pregnancy age; LPA: Large for pregnancy age; SPA: Small for pregnancy age.

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INTRODUCTION

The use of phytochemical components in therapy or complementary medicine is an issue of growing interest, especially in developing countries in Asia and Africa.[1] Maternity care is a field in which complementary medicine use has attracted attention in public health and research communities, due to the increased use of herbal drugs by pregnant females.[2] Globally, epidemiological studies suggest that

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pregnant women herbal drug utilization rates are 51.4% in Eastern Asia, up to 60% in Western nations, and reaching 90.3% in Africa.[1,3,4] Despite the high utilization and adherence of herbal drugs by pregnant females, some concerns are raised concerning their appropriate quality control, mechanisms, efficacy, and possible prenatal toxicities.[5]

Extraction of herbal medicine is one of the important processes in preparing herbal products of high quality. Conventional methods of extraction, including solid–liquid extraction (SLE) and ultrasound (US) extraction, are laborious and time-consuming.[6] Finding new methods of extraction is of growing importance. Infrared-assisted extraction (IR-AE) is a new and promising method in preparing phytochemical products of good quality in a timely and cost-effective manner. The principle of IR-AE is to apply IR radiation that penetrates the samples and extract bioactive compounds promptly utilizing an appropriate solvent.[7] Moreover, studying the factors affecting IR-AE is crucial in the optimization of IR-AE. Optimization of IR-AE for a specific group of bioactive compounds has the dominance in increasing extraction rate and improving product quality with shorter extraction time.

Saussurea lappa (*S. lappa, Asteraceae*) is one of the medicinal plants of high therapeutic potentials. *S. lappa* roots have been used in complementary medicine for its effects against many inflammatory and metabolic disorders including indigestion, colic, and cholecystitis.[8] *S. lappa* is considered a rich source of several polyphenolics, alkaloids, and sesquiterpene lactones,[9-11] with lactones reported to have several biological potentials including antiproliferative and immunomodulatory potentials, although their efficient extraction, isolation, and quantification of these bioactive lactones are very limited.[8]

Gestational diabetes (GDM) is a type of diabetes mellitus that affects globally one in seven pregnant females. GDM is marked by hyperglycemia diagnosed in the second or third trimester of pregnancy, coupled with high risk of adverse perinatal outcomes, including decreased infant birth weight, increased risk of preeclampsia and preterm delivery, and increased the chances of cesarean delivery.[12] Hyperglycemia provokes high oxidative stress which contributes to GDM pathology and complications.[13]

Several conventional drugs have been utilized in the management of diabetes although ideal glycemic control is seldom achieved.[14] Medicinal herbs have been utilized widely for control of many kinds of diabetes and its complications.[15-17] Diabetic women conventionally use aqueous extracts of medicinal plants during pregnancy for their hypoglycemic potentials and higher patients' adherence;[14] however, more studies should be conducted to prove the safety and the efficacy of these complementary phytochemicals.[18]

Although *S. lappa* was not widely discovered for its hypoglycemic potentials, *S. lappa* is considered one of the good candidates to investigate its potentials against GDM, due to its bioactive lactones immunomodulatory potentials and good safety history. Optimization of *S. lappa* extraction and fractionation procedures for improved extraction and isolation of the bioactive lactones would positively enhance the chances of better exploring *S. lappa* and its most active compounds with anti-GDM potentials and their possible mechanisms of actions.

Therefore, the aim of this study is to optimize a rapid and efficient IR-AE method for *S. lappa* bioactive constituents, phytochemically investigate its content, fractionate, and isolate its most active constituent and to assess its biological effects against GDM.

MATERIALS AND METHODS

Plant materials and chemicals

S. lappa roots have been commercially obtained from Mecca herbalist (Marsa-Matrouh, Egypt). The roots were authenticated using a reference sample. A representative sample was kept in the faculty herbarium labeled by a voucher specimen number (PS-17-05) for future reference. All chemicals, solvents, and standards used in this study were of analytical grade obtained from Sigma-Aldrich (Germany) and utilized without additional purification.

Optimization of infrared-assisted extraction

The powder of *S. lappa* was extracted by an IR-AE apparatus[7] using an aqueous solution. The factors which influence IR-AE performance were studied and optimized to maintain reliable quality and high performance. The IR extraction ceramic IR transmitter was used to adjust the IR power which was controlled by a proportional–integral–derivative controller and an automatic/manually temperature control system, to heat the solvent matrix in the IR-AE flask and its distance from the ceramic transmitter is changeable.[7] The specific IR energy $W_{IR}\left(\frac{\text{kJ}}{\text{kg}}\right)$ was calculated as follows:

$$W_{IR} = \frac{P_{IR} \times t_{IR}}{m}$$

Where P is the power of the generator, t is the total treatment duration (s), and m is the product mass (kg). Furthermore, to compare the efficiency of extraction, traditional SLE and US methods were done. SLE method was done for *S. lappa* (50 g, 70 mesh particle size) and aqueous solvent 250 ml for 120 min to obtain the *S. lappa* SLE extract (SL-SLE). In addition, the US method also utilized 50 g, 70 mesh particle size powder, and 250 ml aqueous solvent for 60 min to obtain *S. lappa* US extract (SL-US). All extracts (SL-IR, SL-US, and SL-SLE) were freeze-dried separately using Edwards's vacuum freeze dryer (Germany). Each extract total yield has been determined by some modifications using a previously described method[19] and was kept under −4°C until further utilization.

Reversed-phase high-performance liquid chromatography standardization of *Saussurea lappa* infrared extract, *Saussurea lappa* ultrasound extract, and *Saussurea lappa* solid–liquid extract

High-performance liquid chromatography (HPLC) conditions were developed using an Agilent HPLC apparatus (Japan) utilizing various stationary phase columns, solvent systems, and wavelengths (200–400 nm). The best chromatographic standardization for *S. lappa* extracts was attained utilizing an RP-C18 Merck end-capped LiChrospher column (250 mm × 4.6 mm I.D.; 5-μM particle size) (Germany) and Milli-Q water (0.05% formic acid) MeOH mixture (47:53, v/v) as a mobile phase with 1 ml/min flow rate at 222 nm and the column temperature was kept at 25°C.

Bioguided fractionation of *Saussurea lappa*, isolation, and ^{1}H, ^{13}C NMR identification of active compounds

The *S. lappa* IR-AE extract (SL-IR) was fractionated by a column chromatography technique utilizing a silica gel column (20 cm × 64 cm) and developed with a gradient Hexane–EtAc 80% EtOH systems (1:0:0, 30:1:0, 20:1:1, 15:1:1, 10:1:1, 8:1:2, 5:1:2, 3:1:2,1:1:1, 0:0:1, v/v/v). Similar fractions were identified through a reversed-phase HPLC (RP-HPLC) method, grouped, and concentrated. Each grouped fraction was further isolated by another column chromatography technique utilizing RP silica gel column (15 cm × 30 cm) and eluted with Milli-Q water EtOH system (3:1, v/v) to obtain four subfractions. Each fraction was

in vivo tested for their potentials toward GDM in a similar way as the tested extracts. The most active fraction was dissolved in dimethyl sulfoxide-d6 and identified utilizing ^{1}H, ^{13}C NMR method using a Bruker ARX-300 spectrometer (Germany).

Animals, gestational diabetes induction, and experimental design

Female virgin albino mice (22–30 g) were obtained from BAU animal house (Lebanon) and were kept under standard laboratory conditions (20°C, alternating 12-h dark/light cycle, water-free access) and standardized food (unless otherwise stated). The mice have been cared for abiding by the principles of the Care Guide and Experimental Animals Use and with the approval of the BAU Institutional Review Board (2018A-0055-P-R-0278).

After 14 days of adaptation, diabetes was induced in mice with alloxan (180 mg/Kg) administered intraperitoneally (IP) as a single dose. After the diabetic state was confirmed (glycemia >200 mg/dL using Merck-glucometers; Germany), female animals were mated with male nondiabetic animals overnight. The day on which a plug was observed in the vaginal smear was marked as zero gestational day.[20] The mice were then distributed in groups (n = 7/group) [Table 1]. Various extracts (SL-IR, SL-US, and SL-SLE) were orally administered every other day, by oral gavage, from day 0 to the 21st pregnancy day. Blood needed for GDM experiments and biochemical profiles were obtained by pricking the tail. Glycemia was monitored preadministration (day 0) and 1, 7, 14, and 21 days postadministration through glucometers, while the maternal weight was recorded on day 0 (preadministration) and day 21 postadministration. The gravid uterus was also weighed and surgically dissected to count live and dead fetuses with male/female percentage ratio determined. The mean fetal body weight of the nondiabetic control group (NDC) was found 1.69 ± 0.04 g. Offspring in the experimental groups whose birth weights were within the 1.65–1.73 g range were marked as adequate for pregnancy age (APA). Those whose weights were >1.7.3 g were marked as large for pregnancy age, while those <1.65 g were marked as small for pregnancy age.[14] For inspection of external anomalies, all newborns were evaluated utilizing a microscope.

Biochemical profile analysis

Insulin serum level

The serum insulin levels have been monitored before and 21 days postadministration with HPLC using a reversed-phase C18 column at 40°C and 1 ml/min flow rate. Milli-Q water (0.1% TFA) ACN gradient mixture (70:30, v/v) for 5 min, followed by (60:40, v/v) for 10 min, was used as a mobile phase at 214 nm.[21]

Serum catalase, glutathione-reduction, and lipid-peroxide levels

Throughout the GDM assessment, serum catalase levels (CAT), glutathione reduction (GSH), and lipid peroxidation, measured as thiobarbituric acid (TBARS) levels, were also monitored before and 21 days postadministration. The CAT levels were measured in kU/I.[22] The TBARS levels were measured by the TBARS test modified from an experiment explained before.[23] Briefly, TBARS (0.8%) was added to 0.2 ml serum, 8.1% SLS, and 20% diluted HAc in Milli-Q water. One hour after heating (95°C) and then cooling, the combination was

Table 1: Protocol of experimental design

Groups	*n*	Tested substance(s)	Description
A. Nondiabetic mice treated or vehicle-treated (NDC) with SL-SLE, SL-US, SL-IR, ATL, or metformin (MTF) during the pregnancy			
I	7	NDC	Nondiabetic mice: Vehicle (sterile cold saline [0.9%]), oral gavages (PO)
II	7	MTF	Nondiabetic mice: MTF 25 mg/kg, PO
III	7	SL-SLE	Nondiabetic mice: SL-SLE 75 mg/kg, PO
IV	7	SL-SLE	Nondiabetic mice: SL-SLE 150 mg/kg, PO
V	7	SL-SLE	Nondiabetic mice: SL-SLE 250 mg/kg, PO
VI	7	SL-US	Nondiabetic mice: SL-US 75 mg/kg, PO
VII	7	SL-US	Nondiabetic mice: SL-US 150 mg/kg, PO
VIII	7	SL-US	Nondiabetic mice: SL-US 250 mg/kg, PO
IX	7	SL-IR	Nondiabetic mice: SL-IR mg/kg, PO
X	7	SL-IR	Nondiabetic mice: SL-IR 150 mg/kg, PO
XI	7	SL-IR	Nondiabetic mice: SL-IR 250 mg/kg, PO
XII	7	ATL	Nondiabetic mice: ATL 15 mg/kg, PO
XIII	7	ATL	Nondiabetic mice: ATL 30 mg/kg, PO
XIV	7	ATL	Nondiabetic mice: ATL 50 mg/kg, PO
B. Diabetic mice treated or vehicle-treated (DC) with SL-SLE, SL-US, SL-IR, ATL, or metformin (MTF) during the pregnancy			
XV	7	DC	Diabetic mice: Vehicle, PO
XVI	7	MTF	Diabetic mice: MTF 25 mg/kg, PO
XVII	7	SL-SLE	Diabetic mice: SL-SLE 75 mg/kg, PO
XVIII	7	SL-SLE	Diabetic mice: SL-SLE 150 mg/kg, PO
XIX	7	SL-SLE	Diabetic mice: SL-SLE 250 mg/kg, PO
XX	7	SL-US	Diabetic mice: SL-US 75 mg/kg, PO
XXI	7	SL-US	Diabetic mice: SL-US 150 mg/kg, PO
XXII	7	SL-US	Diabetic mice: SL-US 250 mg/kg, PO
XXIII	7	SL-IR	Diabetic mice: SL-IR mg/kg, PO
XXIV	7	SL-IR	Diabetic mice: SL-IR 150 mg/kg, PO
XXV	7	SL-IR	Diabetic mice: SL-IR 250 mg/kg, PO
XXVI	7	ATL	Diabetic mice: ATL 15 mg/kg, PO
XXVII	7	ATL	Diabetic mice: ATL 30 mg/kg, PO
XXVIII	7	ATL	Diabetic mice: ATL 50 mg/kg, PO

SL: *Saussurea lappa*; SL-IR: *Saussurea lappa* infrared extract; SL-US: *Saussurea lappa* ultrasound extract; SL-SLE: *Saussurea lappa* solid-liquid extract; ATL: Alantolactone; NDC: Nondiabetic control; DC: Diabetic control; MTF: Metformin

extracted with IP alcohol MeOH mixture (15:1, v/v), and then, at 532 nm, the UV absorption was read utilizing JASCO spectrophotometer.[23] Furthermore, GSH (μg/mg) levels were also observed by a previously described method.[24]

Statistical analysis

The data were interpreted as mean ± standard error of the mean and were statistically analyzed utilizing Kruskal–Wallis test, proceeded by Dunn's test, for the number of live fetuses and fetal weight observations. For glycemia, biochemical parameters, and maternal weight gain, one-way ANOVA followed by Bonferroni test was utilized. The percentages have been determined utilizing the Fisher's exact test.[14] Statistical differences ($P < 0.05$) have been considered significant.

RESULTS

Optimization of infrared-assisted extraction

To quantitatively extract lactones from *S. lappa*, IR-AE is an interesting technique. Compared with the traditional SLE and US methods, IR-AE has more significant advantages, and it has shown to be a novel and a more efficient method of extraction of bioactive lactones. The factors which influence IR-AE performance have been studied, including solvent concentration, extraction time, powder size, and IR power. The effects of different IR-AE factors were investigated, and optimization of the IR-AE method was done. The best extraction results have shown that the solvent is one of the main factors in the extraction process. As the concentration of the aqueous phase elevated to 20%, the lactones yield reached a maximum and declined afterward. In addition, when the extraction time increased more than 60 min, the yield maintained a steady state. Nevertheless, the more prolonged the extraction time, more damage might affect the targeted components; therefore, 60 min of extraction time was selected. The effect of powder size has also been examined. It was found that 70 mesh powder size was the optimum size for obtaining the highest yield. As >70 mesh powder size led to decrease the surface area and resulted in lower yield, <70 mesh powder size decreased the yield, due to the apparent increase in agglomeration. The effect of IR power on the yield of bioactive lactones has been also studied. The IR extraction ceramic IR transmitter was used to adjust the IR power (63–170 W) which was controlled by a proportional–integral–derivative controller and an automatic/manual temperature control system. The leading extraction results have shown that the IR power is one of the major factors in the extraction process. IR power adjusted to (70 W) has shown the most prominent bioactive lactone extraction as this comparatively low power has decreased the damage to the extract active components. The yielded *S. lappa* extract by the optimized IR-AE was designated by SL-IR.

Furthermore, to compare the efficiency of extraction, traditional SLE and US methods were done. The main disadvantages of the conventional method are the low extraction yield and long extraction time.[25] The various methodological conditions and their corresponding outcomes are summed up in Table 2.

SLE method was done by the same amount of powdered *S. lappa* (50 g, 70 mesh particle size) and aqueous solvent 250 ml as the optimized IR-AE, and the best extraction time was 120 min to obtain the *S. lappa* SLE extract (SL-SLE). In addition, the US method also utilized 50 g, 70 mesh particle size powder, and 250 ml aqueous solvent, but the best extraction time was obtained after 60 min for *S. lappa* US extract (SL-US).

Concerning the yield, the most superior results have been obtained by the optimized IR-AE method, which has given the highest yields by 2.7-fold increase for SL-IR, when compared to the SL-SLE method, and 1.6-fold increase for SL-IR, when correlated to the SL-US technique [Table 2].

Thus, the optimized IR-AE technique has shown to be of higher efficiency and time-saving when compared to conventional methods.

Reversed-phase high-performance liquid chromatography standardization of *Saussurea lappa* infrared extract, *Saussurea lappa* ultrasound extract, and *Saussurea lappa* solid-liquid extract

To standardize and monitor the effects of optimization of the IR-AE method, over US and SLE conventional methods, gradient chromatographic systems were developed and modified for RP-HPLC system. The percentages of the identified compounds were obtained by comparing both RT and spectral results obtained from various extracts and the chromatograms of standard mixtures. Four major lactones were identified in SL-IR, including costunolide (8.6%), dehydrocostuslactone (18.5%), isoalantolactone (20.5%), and alantolactone (ATL) (22.5%) [Figure 1]. For SL-US and SL-SLE, the four major lactones were also identified but with lower concentration, where SL-US major peaks included costunolide (4.9%), dehydrocostuslactone (8.5%), isoalantolactone (21.0%), and ATL (13.9%) [Figures 1 and 2]. For SL-SLE, the major components included costunolide (3.8%), dehydrocostuslactone (1.8%), isoalantolactone (6.3%), and ATL (11.2%) [Figures 1-3].

When compared to conventional methods, the optimized IR-AE method has shown to be a better extraction method maintaining higher quality and quantity of bioactive constituents.

Bioguided fractionation of *Saussurea lappa*, isolation, and ^{1}H, ^{13}C NMR identification of active compounds

By following the bioguided fractionation procedure, the most active fraction of SL-IR has been identified utilizing an NMR spectrometer [Table 3] and animal *in vivo* models, where ^{1}H NMR and ^{13}C NMR analysis have shown that the most active constituent was ATL [Figure 2]. Various elution mixtures utilizing different column chromatography methods were used to quantitatively isolate ATL.[26] Thus, ATL has been tested the same way as SL-IR, SL-US, and SL-SLE to explore their potentials against GDM.

Effects of *Saussurea lappa* infrared extract, *Saussurea lappa* ultrasound extract, *Saussurea lappa* solid–liquid extract, and alantolactone on gestational diabetes

The NDC mice demonstrated a blood glucose level (BGL) <100 mg/dL [Figure 3]. On the other hand, the pregnant diabetic control (DC) group

Table 2: Comparison of *Saussurea lappa* yields (%) between the conventional solid-liquid extract, ultrasound extract, and the infrared-assisted extraction methods

Extraction method	Extract	Initial weight (g)	Extraction time (min)	Solvent volume (ml)	Yield (%)	Lactones (%)	ATL (%)
SLE	SL	50	120	250	4.10	23.1	11.2
US	SL	50	60	250	6.90	48.3	13.9
IR-AE	SL	50	60	250	11.10	70.1	22.5

SL: *Saussurea lappa*; ATL: Alantolactone; IR-AE: Infrared-assisted extraction; US: Ultrasound extract; SLE: Solid-liquid extract

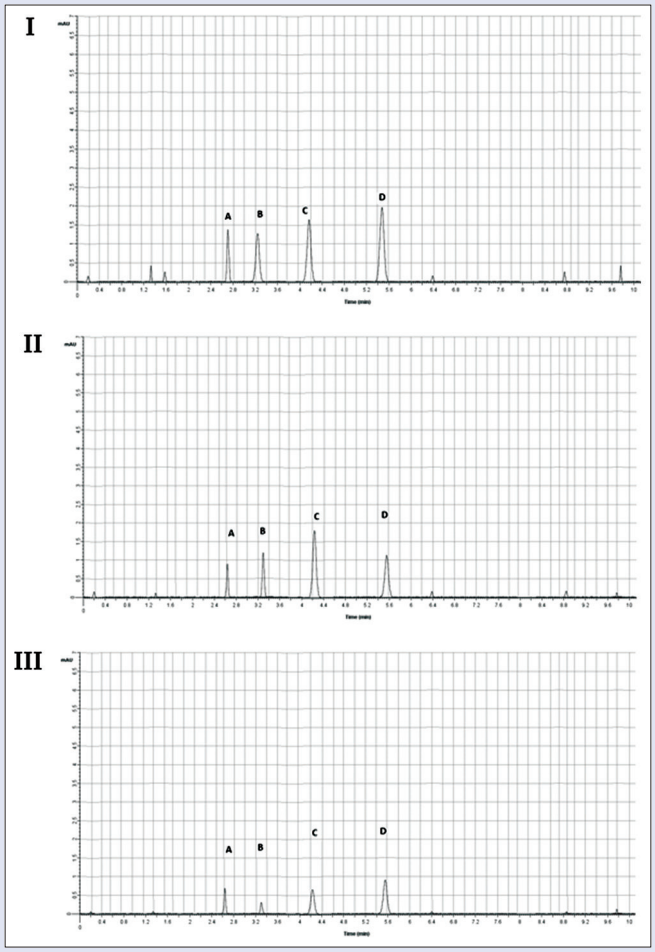

Figure 1: High-performance liquid chromatography analysis *Saussurea lappa* (SL): (i) *Saussurea lappa* solid–liquid extract, (II) *Saussurea lappa* ultrasound extract, and (III) *Saussurea lappa* infrared extract and the major peaks are (A) costunolide, (B) dehydrocostuslactone, (C) isoalantolactone, and (D) alantolactone

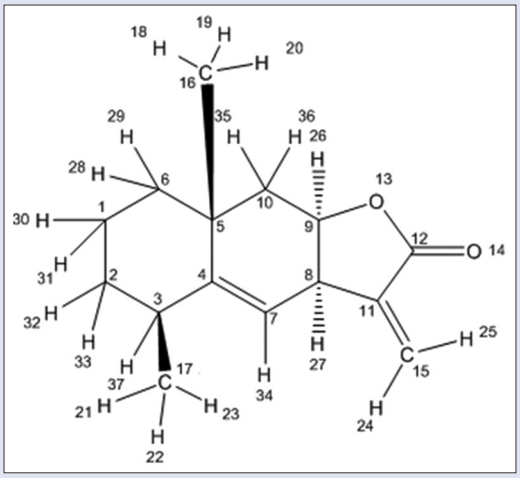

Figure 2: Alantolactone chemical structure

Table 3: Alantolactone ^{1}H -NMR and ^{13}C- NMR data

Position*	δC	Position*	δH, m, (*J* in Hz)
1	22.3	18	1.54, dddd (*J*=13.32, 10.00, 8.93, 1.85 Hz)
2	30.2	19	1.21, s
3	32.4	20	1.82, ddd (*J*=13.17, 5.68, 1.37 Hz)
4	147.1	21	1.02, d (*J*=6.90 Hz)
5	36.4	22	0.99, d (*J*=6.90 Hz)
6	40.8	23	0.98, d (*J*=6.90 Hz)
7	130.8	24	5.57, d (*J*=3.25 Hz)
8	41.1	25	6.15, d (*J*=3.30 Hz)
9	79.7	26	4.40, ddd (*J*=10.00, 8.93, 1.89 Hz)
10	42.3	27	3.20, dd (*J*=10.00, 6.90 Hz)
11	133.2	28	1.82, ddd (*J*=13.20, 5.70, 1.40 Hz)
12	170.1	29	3.00, s
13	-	30	1.50, dddd (*J*=13.32, 10.00, 8.93, 1.85 Hz)
14	-	31	1.54, dddd (*J*=13.32, 10.00, 8.93, 1.85 Hz)
15	121.2	32	1.54, dddd (*J*=13.32, 10.00, 8.93, 1.85 Hz)
16	23.1	33	1.49, dddd (*J*=13.30, 5.55, 4.10, 1.91 Hz)
17	18.6	34	5.30, d (*J*=6.90 Hz)
		35	1.20, s
		36	1.90, dd (*J*=15.10, 1.90 Hz)

*Position refers to [Figure 2]

has shown BGL >200 mg/dL [Figure 4]. Metformin 25 mg/Kg (MTF) has been utilized in this study as a positive control. MTF has demonstrated both serious hypoglycemic effects nondiabetic and diabetic pregnant groups [Figures 3 and 4]. Compared to NDC, the administration of SL-IR (75, 150, or 250 mg/Kg), SL-US (75, 150, or 250 mg/Kg), SL-SLE (75, 150, or 250 mg/Kg), or ATL (15, 30, or 50 mg/Kg) did not cause severe hypoglycemia with the nondiabetic group [Figure 3]. In contrast, the administration of SL-IR (75, 150, or 250 mg/Kg), SL-US (75, 150, or 250 mg/Kg), SL-SLE (75, 150, or 250 mg/Kg), or ATL (15, 30, or 50 mg/Kg) showed a significant ($P < 0.05$) normalization to the BGL of the pregnant diabetic-treated group, in a dose-dependent manner, after 21 days treatment, when compared to DC [Figure 4]. However, SL-IR (75, 150, and 250 mg/Kg) showed 47.6%, 56.2%, and 64.3% decrease in BGL in the pregnant diabetic group, after 21 days treatment, when compared to DC, respectively [Figure 4]. Moreover, SL-US (75, 150, and 250 mg/Kg) demonstrated 42.8%, 48.6%, and 56.1% decline in pregnant diabetic mice BGL, respectively, when compared to DC, after 21 days postadministration [Figure 4]. When correlated to DC, SL-SLE (75, 150, and 250 mg/Kg) presented a decrease of 49.5%, 50.5%, and 51.4% in pregnant mice BGL, 21 days posttreatment, respectively [Figure 4]. Furthermore, ATL (15, 30, or 50 mg/Kg) has shown a decline of 41.4%, 47.1%, and 53.3% in BGL, respectively, 21 days postadministration [Figure 4].

Biochemical profile analysis

Insulin serum level

The serum insulin levels have been monitored before and for 21 days posttest administration utilizing an HPLC technique to explore the mechanism of hypoglycemia. Insulin serum level has significantly decreased in NDC group from 1.25 ± 0.02 μg/L to 0.51 ± 0.01 μg/L in pregnant DC group, before treatment (day 0) [Table 4]. Twenty-one days post-SL-IR (75, 150, or 250 mg/Kg) administration, serum insulin levels have increased by 2.6, 2.9, and 3.5 folds, respectively, in pregnant diabetic mice when compared to DC. When correlated with DC, the administration of SL-US (75, 150, or 250 mg/Kg) has elevated serum insulin levels by 2.5, 2.6, and 2.7 folds, respectively, while those of SL-SLE (75, 150, or 250 mg/Kg) increased serum insulin levels by 2.4, 2.5, 2.6 folds, respectively, 21 days postadministration to pregnant diabetic mice [Table 4]. The MTF group (positive control) did not show a significant elevation in serum insulin levels [Table 4], while ATL (15, 30, or 50 mg/Kg) has shown a significant increase in insulin serum level in diabetic pregnant mice by 2.4%, 2.6%, and 2.7%, respectively, when compared to DC, 21 days postadministration [Table 4].

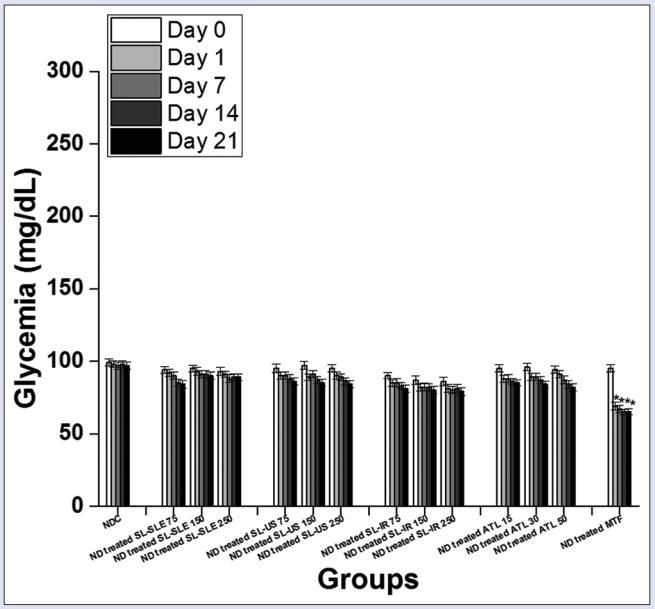

Figure 3: Glycemia on days 0, 1, 7, 14, and 21 of nondiabetic mice treated or nontreated (nondiabetic control) with *Saussurea lappa* solid–liquid extract, *Saussurea lappa* ultrasound extract, *Saussurea lappa* infrared extract, alantolactone, or metformin 25 mg/Kg during the pregnancy (mean ± standard error of the mean, *n* = 7/group). "*" designates the significant results ($P < 0.05$) compared to nondiabetic control group

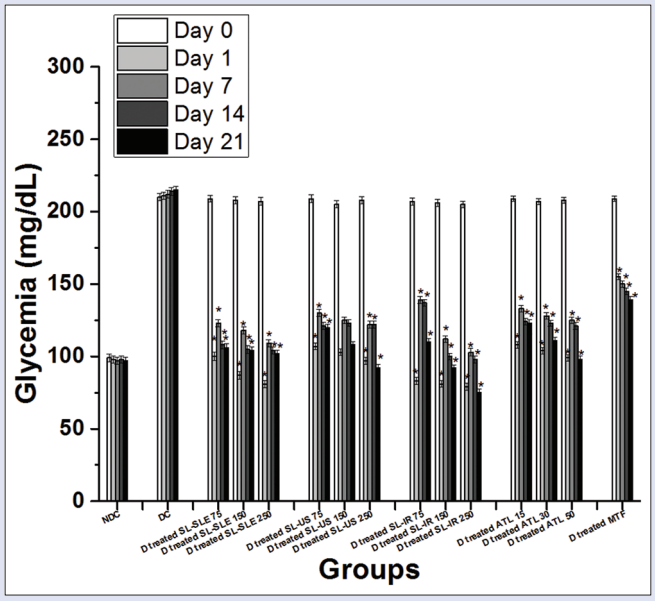

Figure 4: Glycemia on days 0, 1, 7, 14, and 21 of Diabetic (d) mice treated or nontreated (diabetic control) with *Saussurea lappa* solid–liquid extract, *Saussurea lappa* ultrasound extract, *Saussurea lappa* infrared extract, alantolactone, or metformin 25 mg/Kg during the pregnancy (mean ± standard error of the mean, *n* = 7/group). "*" designates the significant results ($P < 0.05$) compared to diabetic control group

Serum catalase, glutathione reduction, and lipid peroxide levels

To understand the hypoglycemic mechanism of the tested compounds, the assessment of the biochemical parameters of the diabetic-treated pregnant groups were studied [Table 1]. The oxidative stress markers, CAT, GSH, and lipid peroxide levels measured as TBARS have demonstrated serious increase in oxidative stress levels in the diabetic groups, before treatment (Predose), when correlated to nondiabetic (normal control) group [Table 5]. The administration of SL-IR (75, 150, or 250 mg/Kg), SL-US (75, 150, or 250 mg/Kg), SL-SLE (75, 150, or 250 mg/Kg), or ATL (15, 30, or 50 mg/Kg) has shown significant ($P < 0.05$) counteracted the hyperglycemia-induced oxidative stress in diabetic pregnant groups, in a dose-dependent manner, after 21 days treatment, when compared to vehicle control group [Table 5]. CAT levels have increased by 0.9, 1.0, and 1.2 folds 21 days post-SL-IR (75, 150, or 250 mg/Kg) administration, respectively, in pregnant diabetic mice when compared to vehicle control. When correlated to vehicle control, the administration of SL-US (75, 150, or 250 mg/Kg) and ATL (15, 30, or 50 mg/Kg) has similarly elevated CAT levels by 0.8, 0.9, and 1.0 folds, respectively, while those of SL-SLE (75, 150, or 250 mg/Kg) increased CAT levels by 0.7, 0.8, and 0.9 folds, respectively, 21 days postadministration to pregnant diabetic mice [Table 5]. The MTF group did not show a significant increase in CAT levels [Table 5]. For TBARS level, SL-IR (75, 150, or 250 mg/Kg) administration has decreased their levels by 59.1, 60.9, and 63.6% 21 days postadministration, respectively, in pregnant diabetic mice when correlated to vehicle control [Table 5]. When compared to vehicle control, the treatment with SL-US (75, 150, or 250 mg/Kg) has decreased TBARS levels by 50.0%, 50.9%, and 53.6%, respectively, while those of SL-SLE (75, 150, or 250 mg/Kg) decreased TBARS levels by 50.9%, 49.1%, and 52.7%, respectively, 21 days postadministration to pregnant diabetic mice [Table 5]. The MTF group did not show a significant decrease in TBARS level [Table 5]. On the other hand, ATL (15, 30, or 50 mg/Kg) has shown a significant decline in TBARS level in diabetic

Table 4: Effects of metformin 25 mg/kg, various doses of *Saussurea lappa* solid-liquid extract, *Saussurea lappa* ultrasound extract, *Saussurea lappa* infrared extract, or alantolactone on serum insulin at predose (day zero) and 21 days postdose

Group	Dose (mg/kg)	Serum insulin (µg/L)	
		Predose	21st day
NDC	-	1.25±0.02	1.26±0.01
DC	-	0.51±0.01	0.28±0.01
MTF[a]	25	0.52±0.02	0.55±0.01
SL-SLE[a]	75	0.52±0.02	1.75±0.04*
SL-SLE[a]	150	0.53±0.01	1.78±0.04*
SL-SLE[a]	250	0.55±0.02	1.80±0.03*
SL-US[a]	75	0.51±0.02	1.78±0.03*
SL-US[a]	150	0.52±0.01	1.85±0.04*
SL-US[a]	250	0.53±0.02	1.88±0.04*
SL-IR[a]	75	0.55±0.01	1.85±0.03*
SL-IR[a]	150	0.53±0.02	2.00±0.04*
SL-IR[a]	250	0.54±0.01	2.30±0.03*
ATL[a]	15	0.50±0.02	1.78±0.03*
ATL[a]	30	0.52±0.01	1.84±0.04*
ATL[a]	50	0.53±0.02	1.86±0.03*

Values represent the mean±SEM (*n*=7). *$P<0.05$ significant from the diabetic vehicle control animals (DC); [a]Compared to vehicle control. SEM: Standard error of mean; SL: *Saussurea lappa*, SL-IR: *Saussurea lappa* infrared extract; SL-US: *Saussurea lappa* ultrasound extract; SL-SLE: *Saussurea lappa* solid-liquid extract; ATL: Alantolactone; NDC: Nondiabetic control; DC: Diabetic control; MTF: Metformin; TBARS: Thiobarbituric acid

pregnant mice by 49.0%, 50.0%, and 51.8%, respectively, when compared to vehicle control, 3 weeks postadministration [Table 5]. GSH levels have increased by 31.2%, 37.3%, and 37.9% 21 days post-SL-IR (75, 150, or 250 mg/Kg) administration, respectively, in pregnant diabetic mice when compared to vehicle control. When correlated to vehicle control,

Table 5: *In vivo* assessment of the antioxidant activities of *Saussurea lappa* extracted by *Saussurea lappa* solid-liquid extract, *Saussurea lappa* ultrasound extract, IR-assisted extraction method (Saussurea lappa infrared extract), or alantolactone on catalase levels in serum, alterations in thiobarbituric acid and reduced reduced glutathione (mean±standard error of mean, *n*=7/group)

Group	Dose (mg/kg)	CAT level (kU/l)		TBARS level (nM/100 g)		GSH (µg/mg)	
		Predose	21st day	Predose	21st day	Predose	21st day
Normal control	-	30.50±1.70	29.98±1.88	0.70±0.04	0.72±0.03	61.40±1.20	61.80±1.90
Vehicle control	-	21.10±1.30	19.70±1.43	1.10±0.05	3.40±0.04	54.63±1.90	45.50±1.80
MTF[a]	25	21.23±1.43	21.98±1.33	1.12±0.03	1.70±0.05	54.10±1.70	53.90±1.60
SL-SLE[a]	75	21.47±1.33	36.33±1.55*	0.94±0.02	0.54±0.03*	57.15±1.80	61.73±1.30*
SL-SLE[a]	150	21.79±1.24	39.11±1.33*	0.98±0.01	0.56±0.04*	56.25±1.70	62.10±1.40*
SL-SLE[a]	250	21.66±2.55	40.16±1.67*	0.96±0.05	0.52±0.07*	58.23±1.40	64.30±1.50*
SL-US[a]	75	22.00±1.76	39.10±1.25*	0.93±0.04	0.55±0.02*	58.14±1.60	63.50±1.80*
SL-US[a]	150	21.10±1.25	41.35±1.90*	0.97±0.03	0.54±0.03*	59.76±1.30	65.80±1.30*
SL-US[a]	250	22.30±1.90	42.74±1.81*	0.92±0.06	0.51±0.04*	59.22±1.40	67.45±1.20*
SL-IR[a]	75	21.23±1.68	40.15±1.65*	1.10±0.02	0.45±0.04*	57.78±1.30	71.70±1.20*
SL-IR[a]	150	21.11±1.33	43.10±1.34*	0.98±0.05	0.43±0.03*	58.95±1.70	74.99±1.30*
SL-IR[a]	250	21.62±1.98	44.80±1.25*	0.93±0.04	0.40±0.01*	57.33±1.90	75.31±1.60*
ATL[a]	15	22.10±1.55	38.10±1.33*	0.96±0.06	0.56±0.05*	56.97±1.80	62.40±1.90*
ATL[a]	30	21.10±1.87	40.93±1.96*	0.97±0.02	0.55±0.04*	58.86±1.30	63.70±1.50*
ATL[a]	50	22.32±1.66	42.55±1.88*	0.94±0.05	0.53±0.07*	58.32±1.60	66.84±1.40*

*P<0.05 significant from the vehicle control animals, [a]Compared to vehicle control. SEM: Standard error of mean; IR-AE: Infrared-assisted extraction; SL: *Saussurea lappa*, SL-IR: *Saussurea lappa* infrared extract; SL-US: *Saussurea lappa* ultrasound extract; SL-SLE: Saussurea lappa solid-liquid extract; ATL: Alantolactone; NDC: Nondiabetic control; DC: Diabetic control; MTF: Metformin; TBARS: Thiobarbituric acid; GSH: Reduced glutathione; CAT: Catalase

the administration of SL-US (75, 150, or 250 mg/Kg) has elevated GSH levels by 16.2%, 20.4%, and 23.5%, respectively, while those of SL-SLE (75, 150, or 250 mg/Kg) increased GSH levels by 13.0%, 13.7%, and 17.7%, respectively, 21 days postadministration to pregnant diabetic mice [Table 5]. Similar to CAT, the MTF group did not show a significant elevation in GSH levels [Table 5], while ATL (15, 30, or 50 mg/Kg) had shown a significant increase in GSH level in diabetic pregnant mice by 14.2%, 16.6%, and 22.4%, respectively, when compared to vehicle control, 21 days postadministration [Table 5].

Reproductive outcomes

To assess the safety of different doses of SL-IR, SL-US, SL-SLE, and ATL on the pregnancy offsprings, various reproductive outcome measures on fetuses have been observed in NDC, nondiabetic-treated groups [Table 6] on the one hand and the DC and the diabetic-treated groups on the other hand [Table 7]. The nondiabetic groups treated with various doses of plant extracts or isolated compound (ATL) did not show significant changes in the parameters of the reproductive outcome when compared to NDC group. The DC group has shown a significant decrease in the maternal weight, the fetal body weight, and APA percentage when correlated with NDC [Table 6]. In contrast, the diabetic-treated groups with various doses of SL-IR, SL-US, SL-SLE, and ATL, especially in the higher doses, have shown a significant increase in the living fetuses, maternal weight, the fetal body weight, and percentage of APA, when correlated to DC group [Table 7]. For the external anomalies, 1.5% of fetuses in the untreated DC group have shown external signs of gastroschisis and exencephaly [Table 7]. The NDC, treated nondiabetic, and diabetic-treated groups have not shown any signs of external anomalies [Tables 6 and 7].

DISCUSSION

Medicinal plants have been used for many years for the management of serious disorders, including all types of diabetes, and have been characterized by higher safety profiles and more patient adherence, when compared to conventional drugs.[27]

S. lappa is one of the medicinal plants that are rich in bioactive compounds, including lactones.[8] Currently, lactones are mainly extracted using solid–liquid or ultrasonic methods,[28] which are laborious and time-consuming. To facilitate the establishment of a comparatively fast and efficient method of extraction of the bioactive lactones, IR-AE was chosen owing to its promising extraction efficiency.[7] To increase the IR-AE efficiency, the factors which influence IR-AE performance were studied. These factors include solvent concentration, extraction time, powder size, and IR power. After studying the IR-AE factors, optimization of the IR-AE method was done. For the solvent concentration, when the concentration of the aqueous phase elevated to 20%, the yield of lactones reached a maximum. In addition, the optimum extraction time was 60 min as the extended extraction time, and the higher IR exposure might affect the bioactive compounds. For the effect of powder size, it was found that 70 mesh powder size was the optimum size for obtaining the highest yield, owing to the increase of the surface area of extraction and the avoidance of the agglomeration. The effect of IR power on the yield of bioactive lactones has also been studied. The leading extraction results have shown when the IR power adjusted to (70 W) and this comparatively low power has the advantage of decreasing the damage to the bioactive components in the *S. lappa* optimized IR-AE extract (SL-IR).

Moreover, to compare the extraction efficiency, SLE and US methods were performed. It was demonstrated that the main disadvantages of these conventional methods are the low extraction yield and long extraction time, as reported before in literature.[25]

The highest yield has been reached by the optimized IR-AE method, which resulted in 2.6 and 1.5 folds increase in the *S. lappa* yield when compared to the SL-SLE method and the SL-US method, respectively. The optimized IR-AE technique has proved to be of higher efficiency and time-saving when compared to conventional methods.

Furthermore, *S. lappa* roots have immunomodulatory effects and have been used in traditional medicine in the management of many disorders such as indigestion, colic, and cholecystitis.[8] GDM is one of the metabolic disorders that develop during pregnancy and marked by hyperglycemia. The high blood sugar levels will affect the pregnant females and may lead to serious complications to both the mother and the offspring, including infant birth weight, infant adiposity, preterm delivery, preeclampsia,

Table 6: Reproductive outcome from nondiabetic mice (nondiabetic) treated or vehicle-treated (nondiabetic control) with *Saussurea lappa* solid-liquid extract, *Saussurea lappa* ultrasound extract, *Saussurea lappa* infrared extract, alantolactone, or metformin during the pregnancy

Doses (mg/kg)/ reproductive outcome	Groups													
	NDC	ND + MTF 25	ND + SL-SLE 75	ND + SL-SLE 150	ND + SL-SLE 250	ND + SL-US 75	ND + SL-US 150	ND + SL-US 250	ND + SL-IR 75	ND + SL-IR 150	ND + SL-IR 250	ND + ATL 15	ND + ATL 30	ND + ATL 50
Pregnant female (*n*)	7	7	7	7	7	7	7	7	7	7	7	7	7	7
Live fetuses (*n*)/L	69	67	65	61	60	63	67	63	68	64	61	64	63	67
Dead fetuses (*n*)/L	1	2	1	0	0	1	0	0	0	0	0	1	0	0
Sex ratio (male/female)	14/52	17/48	19/45	11/50	20/40	29/33	19/48	34/29	41/27	25/39	37/24	24/20	24/39	32/35
Maternal weight (g)[b]	29.50±2.40	26.5±2.40*	28.40±2.20	28.90±1.90	30.50±2.20	29.70±1.80	30.20±2.10	30.70±2.50	30.10±1.90	30.80±2.10	31.10±2.60	28.60±1.60	29.70±1.40	29.90±1.70
Fetal body weight (g)														
Mean±SEM[a]	1.69±0.04	1.54±0.03*	1.71±0.02	1.72±0.03	1.73±0.01	1.66±0.02	1.67±0.04	1.68±0.03	1.68±0.03	1.69±0.02	1.70±0.03	1.70±0.02	1.71±0.03	1.73±0.03
SPA fetuses (%)[b,**]	21.5	28.5	22.5	15.8*	14.8*	18.7	14.7*	16.3*	19.5	18.1	16.9	19.5	19.0	18.5
APA fetuses (%)[b,***]	49.2	42.6*	60.4*	76.4*	77.8*	61.7*	78.3*	77.5*	62.7*	77.5*	80.8*	57.5	60.4*	62.6*
LPA fetuses (%)[b,****]	29.3	28.9	17.1*	7.8*	7.4*	19.6*	7.0*	6.2*	17.8*	4.4*	2.3*	23.0	20.6*	18.9*
External anomalies														
Number of fetuses examined (litter)	69	67	65	61	60	63	67	63	68	64	61	64	63	67
Total number of fetuses (%) with alteration	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gastroschisis	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Exencephaly	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Data shown as mean±SEM and proportions (%). *P<0.05 compared to nondiabetic control Bonferroni; [a]Kruskal-Wallis test; [b]Fisher exact test. **APA; ***LPA; ****SPA. SEM: Standard error of mean; IR-AE: Infrared-assisted extraction; SL: *Saussurea lappa*; SL-IR: *Saussurea lappa* infrared extract; SL-US: *Saussurea lappa* ultrasound extract; SL-SLE: *Saussurea lappa* solid-liquid extract; ATL: Alantolactone; NDC: Nondiabetic control; DC: Diabetic control; MTF: Metformin; TBARS: Thiobarbituric acid; GSH: Reduced glutathione; CAT: Catalase; APA: Adequate for pregnancy age; LPA: Large for pregnancy age; SPA: Small for pregnancy age

Table 7: Reproductive outcome from diabetic mice (D) treated or vehicle-treated (nondiabetic) with *Saussurea lappa* solid-liquid extract, *Saussurea lappa* ultrasound extract, *Saussurea lappa* infrared extract, alantolactone, or metformin during the pregnancy

Doses (mg/kg)/ reproductive outcome	Groups													
	DC	D + MTF 25	D + SL-SLE 75	D + SL-SLE 150	D + SL-SLE 250	D + SL-US 75	D + SL-US 150	D + SL-US 250	D + SL-IR 75	D + SL-IR 150	D + SL-IR 250	D + ATL 15	D + ATL 30	D + ATL 50
Pregnant female (*n*)	7	7	7	7	7	7	7	7	7	7	7	7	7	7
Live fetuses (*n*)/L	66	65	63	59	58	61	65	62	65	62	59	62	61	65
Dead fetuses (*n*)/L	2	3	2	0	0	2	0	0	1	0	0	2	0	0
Sex ratio (male/female)	19/47	22/43	25/38	16/43	15/35	34/27	25/43	28/34	33/32	32/30	30/29	27/34	29/32	32/30
Maternal weight (g)[b]	25.10±1.60	22.50±1.80*	24.14±1.40	26.40±1.60	27.60±1.40*	25.80±1.30	26.10±1.10	28.90±1.30*	25.80±1.70	28.60±1.30*	29.80±1.60*	24.30±1.70	25.40±1.90	26.90±1.40*
Fetal body weight (g)														
Mean±SEM[a]	1.50±0.03	1.37±0.01*	1.54±0.02	1.59±0.01*	1.61±0.02*	1.49±0.02	1.60±0.03*	1.62±0.02*	1.55±0.02	1.62±0.01*	1.70±0.02*	1.55±0.01	1.57±0.02	1.59±0.01*
SPA fetuses (%)[b,**]	18.7	24.7*	19.5	13.7*	12.9*	16.3*	12.8*	14.2*	17.0*	15.7*	14.7*	16.9*	16.5*	16.1*
APA fetuses (%)[b,***]	46.2	40.0	56.7*	71.8*	73.1*	58.0*	73.6*	72.9*	58.9*	72.9*	77.1*	54.1*	56.8*	58.8*
LPA fetuses (%)[b,****]	35.1	35.3	23.8*	14.5*	14.0*	25.7*	13.6*	12.9*	24.1*	11.4*	8.2*	29.0*	26.7*	25.1*
External anomalies														
Number of fetuses examined (litter)	66	65	63	59	58	61	65	62	65	62	59	62	61	65
Total number of fetuses (%) with alteration	1 (1.5%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gastroschisis	1 (1.5%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Exencephaly	1 (1.5%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Data shown as mean±SEM and proportions (%). *P<0.05 compared to diabetic control (DC) Bonferroni; [a]Kruskal-Wallis test; [b]Fisher's Exact Test. **APA; ***LPA; ****SPA; SEM: Standard error of mean; IR-AE: Infrared-assisted extraction; SL: *Saussurea lappa*; SL-IR: *Saussurea lappa* infrared extract; SL-US: *Saussurea lappa* ultrasound extract; SL-SLE: Saussurea lappa solid-liquid extract; ATL: Alantolactone; NDC: Nondiabetic control; DC: Diabetic control; MTF: Metformin; TBARS: Thiobarbituric acid; GSH: Reduced glutathione; CAT: Catalase; APA: Adequate for pregnancy age; LPA: Large for pregnancy age; SPA: Small for pregnancy age

and high C-section delivery rates.[12] In the current study, the optimized IR-AE extract of *S. lappa* and its most bioactive compound was tested for their potentials against GDM.

To phytochemically investigate *S. lappa* various extracts (SL-SLE, SL-US, and SL-IR) and to explore their most active constituent (s), RP-HPLC method coupled with bio-guided fractionation and isolation procedure using ^{1}H and ^{13}C NMR method has been utilized in alloxan-induced GDM in a mouse model. Four major lactones were identified in SL-IR, including costunolide, dehydrocostuslactone, isoalantolactone, and ATL, where ATL was shown to be the most active lactone in the optimized SL-IR extract. The isolated ATL was tested the same way as the SL-IR, SL-US, and SL-SLE extracts for its anti-GDM potentials.

Treatment with various doses of SL-IR, SL-US, SL-SLE, or ATL did not show serious hypoglycemia in the nondiabetic group, as an advantage over the positive control, MTF which demonstrated severe hypoglycemia in the nondiabetic group. However, different doses of SL-IR, SL-US, SL-SLE, or ATL have shown significant ($p < 0.05$) and dose-dependent decrease in glycemia in the pregnant diabetic group, when compared to DC. The highest dose of SL-IR (250 mg/Kg) has shown normalization of blood glucose level in the pregnant diabetic group, when correlated to other groups, including the MTF-treated group. This shows that the optimized IR-AE extraction is the most efficient method of extraction and that SL-IR extract is capable of managing GDM, with superior results to the conventionally used drug, MTF.

To explore SL-IR anti-GDM mechanism, insulin serum levels and the *in vivo* oxidative stress markers, CAT, GSH, and TBARS levels have been monitored for the gestational period (21 days) for all pregnant diabetic groups. SL-IR has shown superiority in insulin secretagogue potentials as well as it *in vivo* antioxidant potentials, by significantly increasing CAT and GSH levels and decreasing TBARS level, especially in its highest dose SL-IR (250 mg/Kg). This indicates that the antioxidant and the insulin secretagogue activities might be among the main mechanisms by which the SL-IR controls GDM.

To assess *S. lappa* extracts (SL-IR, SL-US, and SL-SLE) and isolated compound (ATL) safety, various reproductive outcome measures on fetuses have been observed in all pregnant nondiabetic and diabetic-treated groups postnatally. The nondiabetic groups treated with various doses of SL-IR, SL-US, SL-SLE, or ATL did not show significant changes in the parameters of the reproductive outcome when compared to the NDC group. The diabetic-treated groups with different doses of SL-IR, SL-US, SL-SLE, and ATL have increased APA levels and did not show any signs of external anomalies. This signifies the high safety profile of *S. lappa* extracts and ATL on the fetus outcomes. In pregnant females with uncontrolled diabetes, fetal anomalies are very frequent.[29] Moreover, hypoinsulinemia has been evident to restrict the fetal growth in diabetic pregnancies.[30] Furthermore, antioxidant enzymes offer protection against hyperglycemia-induced oxidative stress malformations.[31] Therefore, the controlling of GDM, oxidative stress reduction, and insulin secretagogue activities might be among the mechanisms by which *S. lappa* decreases offspring anomalies.

In the current study, it is the first report in which IR-AE technique has been optimized for bioactive lactones extraction from *S. lappa*. The RP-HPLC method was coupled with bioguided fractionation and isolation of the most active lactone (ATL) utilizing ^{1}H and ^{13}C NMR in alloxan-induced GDM in mice model. The optimized IR-AE technique has shown to be a rapid and efficient method of extraction. The SL-IR has shown superiority in controlling GDM for pregnant groups with high safety profile on the offspring. Reduction of hyperglycemia-induced oxidative stress and insulin secretagogue activities might be among the mechanisms by which *S. lappa* decreases offspring anomalies and controls GDM for further clinical studies.

CONCLUSION

It is the first report in which IR-AE technique has been optimized for bioactive lactones extraction from *S. lappa*. The RP-HPLC method was coupled with bio-guided fractionation and isolation of the most active lactone (ATL) utilizing ^{1}H and ^{13}C NMR in alloxan-induced GDM in mice model. The optimized IR-AE technique has shown to be a rapid and efficient method of extraction. The SL-IR has shown superiority in controlling GDM for pregnant groups with high safety profile on the offspring. Reduction of hyperglycemia-induced oxidative stress and insulin secretagogue activities might be among the mechanisms by which *S. lappa* decreases offspring anomalies and controls GDM for further clinical studies.

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Authors contribution

KR (main author) did most of the experimental part, analyzed the data, wrote and revised the manuscript. FS and ND helped in writing and revising the manuscript. HR and NL did some experiments and revised the manuscript.

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Conflicts of interest

There are no conflicts of interest.

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