



Impact of extraction mode on phytoconstituents and antidiabetic activity of extracts of *Calotropis procera* (Aiton) Dryand.

[Impacto del modo de extracción en los fitoconstituyentes y la actividad anti-diabética de los extractos de *Calotropis procera* (Aiton) Dryand.]

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Abstract

Context: The extraction mode plays a key role in extracting a particular type of pharmacologically active compounds that can be isolated as leads to develop novel therapeutic molecules.

Aims: To evaluate the impact of extraction mode on extract yield, phytochemicals constituents, and antidiabetic activity of different parts of the *Calotropis procera*.

Methods: The different parts extracts were prepared by maceration and Soxhlet extraction. The resulting extracts were subjected to determining phytochemical constituents and antidiabetic activity using *in vitro* models (inhibition of alpha-amylase, glucose uptake by yeast cell, and inhibition of non-enzymatic Hb-glycation).

Result: The yield of extracts was higher in Soxhlet extraction in both solvents compared to maceration. Likewise, methanol extract of all the parts prepared by Soxhlet extraction exhibited higher contents of total polyphenols and tannins (430.2 ± 0.99 and 228.3 ± 3.8 , respectively). On the other hand, total flavonoids and terpenoids were more in all parts prepared by maceration. The extract of flower and stem prepared by maceration exhibited $64.87 \pm 0.720\%$, $70.32 \pm 0.75\%$, and $56.86 \pm 0.13\%$ antidiabetic activity in alpha-amylase enzyme, non-enzymatic Hb glycation inhibition, and glucose enhancement in yeast, respectively ($p < 0.05$) as compared to standards. The IC_{50} of alpha-amylase inhibitory assay and non-enzymatic Hb-glycation was $600 \mu\text{g/mL}$ and $420 \mu\text{g/mL}$, respectively, and EC_{50} for glucose uptake assay was $400 \mu\text{g/mL}$.

Conclusions: This study revealed that maceration extracts with higher flavonoid and terpenoid content have higher antidiabetic action than Soxhlet extracts. This study will assist in the future for the isolation of antidiabetic compounds from the plant.

Keywords: antidiabetic activity; *Calotropis procera*; maceration; phytochemical constituents; Soxhlet extraction.

Resumen

Contexto: El modo de extracción juega un papel clave en la extracción de un tipo particular de compuestos farmacológicamente activos que pueden aislarse como conductos para desarrollar nuevas moléculas terapéuticas.

Objetivos: Evaluar el impacto del modo de extracción en el rendimiento del extracto, los componentes fitoquímicos y la actividad anti-diabética de diferentes partes de *Calotropis procera*.

Métodos: Los extractos de las diferentes partes se prepararon mediante maceración y extracción Soxhlet. Los extractos resultantes se sometieron a la determinación de constituyentes fitoquímicos y actividad anti-diabética utilizando modelos *in vitro* (inhibición de alfa amilasa, captación de glucosa por células de levadura e inhibición de glicación de Hb no enzimática).

Resultados: Se encontró que el rendimiento de extractos fue mayor en la extracción Soxhlet en ambos solventes en comparación con la maceración. Asimismo, el extracto metanólico de todas las partes elaborado por extracción Soxhlet presentó mayores contenidos de polifenoles totales y taninos (430.2 ± 0.99 and 228.3 ± 3.8 , respectivamente). Por otro lado, los flavonoides totales y terpenoides fueron más en todas las partes, preparados por maceración. El extracto de flor y tallo preparado por maceración exhibió $64,87 \pm 0,720\%$, $70,32 \pm 0,75\%$ y $56,86 \pm 0,13\%$ de actividad anti-diabética en la enzima alfa amilasa, inhibición de la glucosilación de Hb no enzimática y mejora de la glucosa en levadura respectivamente ($p < 0,05$) en comparación a los estándares.

Conclusiones: Este estudio reveló que los extractos de maceración con mayor contenido de flavonoides y terpenoides tienen mayor acción anti-diabética que los extractos de Soxhlet. Este estudio ayudará en el futuro a aislar compuestos anti-diabéticos de plantas.

Palabras Clave: actividad anti-diabética; *Calotropis procera*; constituyentes fitoquímicos; extracción Soxhlet; maceración.

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INTRODUCTION

The yield and chemical constituents of extracts depend on the mode of extraction and solvents used (Abubakar and Haque, 2020). Maceration and Soxhlet extractions are the most commonly used for extracting plant materials (Abubakar and Haque, 2020). The former is usually carried out at ambient temperature to extract constituents in their original form, whereas later, the solvent is evaporated and condensed repeatedly. Heating may damage heat-labile compounds (Zhang et al., 2018). Therefore, the present study aimed to use different extraction modes to investigate their impact on yield, phytochemical constituents, and pharmacological activity of a traditional medicinal plant.

Calotropis procera (Family: *Apocynaceae*), commonly known as 'akk', is a wildy grown plant. It has been used in Ayurvedic, Unani, Arabic, and traditional Sudanese systems of medicine for the treatment of various ailments (Sudesh et al., 2012). Almost all parts of the plant have been investigated for pharmacological activities (Rohit et al., 2012). Different extracts of the stem have shown antiproliferative effects (Magalhaes et al., 2010), anti-inflammatory and gastromucosal protective effects (Tour and Talele, 2011). The root extracts have exhibited anticonvulsant (Jalalpure, 2009) and antifertility activity (Kamath and Rana, 2002). The root extracts were reported to reduce blood glucose levels in streptozotocin-induced diabetic rats (Bhaskar and Ajay, 2009). The flower extracts have shown promising molluscicidal activity (Larhsini et al., 1997) and hepatoprotective effect (Setty et al., 2007). The leaf extracts have shown potent anti-inflammatory and analgesic (Saba, 2011), antiulcer and anticancer activities (Al-Taweel et al., 2017). The significant *in vitro* and *in vivo* antidiabetic activity of leave extract has been reported (Neto et al., 2013; Kazeem et al., 2016). These pharmacological activities indicate the medicinal significance of different parts of the plant. Despite all such activities, none of the studies was found indicating the effect of extraction on chemical constituents and antidiabetic activity. Such investigation may add value to the commercial and medicinal significance of the plant. Therefore, this study aimed to investigate the effect of extraction methods on yield, phytochemical constituent, and antidiabetic activity of different parts of *Calotropis procera*.

MATERIAL AND METHODS

Plant material collection and authentication

The flower, leaf, root, and stem of the plant were collected from different areas of Lahore, Pakistan, from April to June 2018. The plant was authenticated by Prof. Dr. Zaheer U-Din Khan, Government College University, Lahore, Pakistan, whereby a specimen voucher bearing number 2986 was submitted. All the parts were cleaned from foreign matter, washed, and dried under shade for a month. All the parts were pulverized to a fine powder and kept separately in cleaned jars for further use.

Chemicals

Aluminum nitrate, potassium acetate, copper sulfate, quercetin, Folin-Ciocalteu (FC) reagent, gallic acid, glucose, tannic acid, sodium carbonate, and perchloric acid were obtained from Merck, Darmstadt, Germany. Alpha-amylase (3-5 U/mg UniChem Chemical Reagent, Beograd), potato starch (Sigma-Aldrich), acarbose (Bayer Pakistan Ltd., Pakistan), hemoglobin powder (HiMedia Laboratories, Pvt, Ltd., India), gentamicin (Merck Pharmaceuticals Ltd., Pakistan), and commercial baker yeast were procured from the local market. In-lab prepared distilled water was used where needed.

Preparation of extracts

The flower, leaf, root, and stem extracts were prepared by cold maceration (CM) and Soxhlet extraction (SE). Forty grams of each part was extracted by maceration for 3 days using 200 mL of hexane and methanol. The procedure was repeated thrice using a fresh solvent. The same quantity of each material was extracted by the Soxhlet apparatus for 72 h using 500 mL of hexane and methanol at 65°C. All the extracts were dried under reduced pressure by a rotatory evaporator. The yield of each extract was calculated with reference to dried material. The extracts were placed in a cool and dry place at 4°C till further use.

Estimation of phytochemical constituents

Total polyphenol

The Singleton et al. (1999) method was used for total polyphenol content determination. Briefly, a reaction

mixture containing 200 μ L test sample/standard and FC reagent was allowed to stand 4 min and mixed with 1 mL of sodium carbonate solution (15%, w/v), and the volume was made 3 mL with methanol. The mixture was incubated for 2 h at room temperature, and the absorbance was measured at 760 nm at UV-Visible spectrophotometer (Model-2550, Shimadzu Scientific Instruments, USA, equipped with operating system UV Probe 2.21). A blank containing all the components and methanol equivalent to sample volume was prepared and treated like the sample. The gallic acid solutions in a concentration range (10, 25, 50, 100, 200 and 400 μ g/mL) were treated as the sample to construct a calibration curve to determine total polyphenols (mg equivalents of gallic acid, mgGAE/g).

Total flavonoids

Total flavonoid content was determined by the method of Chang et al. (2002). A reaction mixture containing 200 μ L of test sample/standard, 100 μ L of $AlCl_3$, 100 μ L of potassium acetate (1 M), and 4.6 mL of distilled water was incubated at room temperature for 45 min in the dark. Then, the absorbance was measured at 415 nm against a blank (UV-Visible spectrophotometer (Model-2550, Shimadzu Scientific Instruments, USA), blank containing all components replacing sample with methanol. The quercetin solutions of different concentrations were treated like the sample to construct a calibration curve to determine total flavonoids (mg equivalents of quercetin, mgQE/g).

Total tannins

Total tannins were estimated using FC reagent with minor modification (Alif et al., 2018). A reaction mixture containing 200 μ L sample/standard solution, 0.5 mL of FC reagent, and 7.5 mL distilled water was kept for 5 min. Then, 1 mL of sodium carbonate solution (30%, w/v) and 10 mL of distilled water were added, and the mixture was incubated for 30 min at the dark. The absorbance was measured spectrophotometrically at 725 nm against a blank containing all the ingredients replacing sample volume with methanol. The absorbance of tannic acid solution (10, 25, 50, 100, 200, 400 μ g/mL) was used to construct a calibration curve. Total tannins (mg equivalents of tannic acid, mgTAE/g) were determined from the linear regression equation.

Total triterpenoids

Total triterpenoid content was determined by the method (Fan and He, 2006). A reaction mixture containing 200 μ L of sample/standard (lupeol), 300 μ L of

(7.5%, w/v) of vanillin glacial acetic acid solution and 2.5 mL of perchloric acid was heated for 30 min at 50°C. The mixture was allowed to cool in ice-cold water. Then, 5 mL of glacial acetic acid were added, and the absorbance was taken spectrophotometrically at 540 nm against blank. The blank containing all the components was prepared replacing sample volume with methanol. The lupeol solutions were treated like the sample to construct a calibration curve to determine total triterpenoids content (mg equivalents of lupeol, mgLE/g).

In vitro antidiabetic activity

Inhibition of alpha-amylase

The alpha-amylase inhibition activity was performed as described by Kazeem et al. (2016). Briefly, a reaction mixture containing 1 mL of alpha-amylase solution (1%) prepared in 0.02 M phosphate buffer solution (pH 6.9) and 1 mL sample (1 mg/mL) was incubated at 37°C for 5 min. Then, 1 mL of 1% starch solution was added, and contents were incubated again at 37°C for 10 min. The reaction was stopped by adding 2.0 mL of 96 mM 3, 5-dinitro salicylic acid and boiling in a water bath for 10 min. The contents were allowed to cool at room temperature, and after adding 10 mL distilled water, the absorbance was measured spectrophotometrically at 540 nm against a blank, containing all the components and methanol in place of the sample. Acarbose was used as a standard, and the enzyme inhibition (EI) was determined using the equation [1].

$$EI (\%) = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \quad [1]$$

Glucose uptake by yeast

Glucose uptake by yeast cells was determined using the method (Cirillo, 1962). A quantity of 1 g of commercial baker yeast was added in distilled water (10 mL), mixed and centrifuged at 4000 rpm for 10 min. The supernatant was removed, and the extraction procedure was repeated till clear supernatant was obtained. The yeast pellet was suspended 10 mL distilled water (1 mL of clear yeast supernatant and 9 mL of distilled water). A reaction mixture containing an equal volume of 10 mM glucose solution and sample/standard (1 mg/mL) was incubated at 37°C for 10 min. Then, 100 μ L yeast suspension was mixed by vortex, and contents were further incubated for 1 h at 37°C. The reaction mixture was centrifuged for 5 min at 2500 rpm, and the quantity of glucose in the supernatant was determined at 540 nm (UV-Visible spectrophotometer (Model 2550, Shimadzu Scientific Instruments, USA, equipped with operating

system UV Probe 2.21). Metformin was used as standard, and blank contained distilled water instead of the sample was used as a blank. The increase in glucose uptake (IGU) was determined using the equation [2]. The EC₅₀ was calculated from a dose-dependent response (50-1000 µg/mL).

$$\text{IGU (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \quad [2]$$

Non-enzymatic hemoglobin glycation inhibition

The non-enzymatic anti-glycation effect was determined (Parker et al., 1981) and modified by (Adisa et al., 2004). The sample/standard, glucose, and hemoglobin (Hb) solutions were prepared in 0.01 M phosphate buffer pH (7.4). A reaction mixture containing equal volume of extract/standard gallic acid (1 mg/mL), glucose 10 mM, Hb (0.6 mg/mL) and gentamicin (0.2 mg/mL) solutions were incubated in the dark at room temperature for 72 h. Then, the absorbance was measured at 443 nm at different time intervals of 24, 48, and 72 h. The blank was prepared in the same way by replacing the sample with buffer. Inhibition of glycosylation of hemoglobin was determined using equation [1]. The IC₅₀ was also determined from the dose-dependent response at 72 h.

Statistical analysis

All the tests were performed in triplicate, and data were expressed as mean ± SD. The data was analyzed by COSTAT 6.451 version, and for comparison, the Student-Newman-Keuls test was used at p<0.05. The p values <0.05 were considered statistically significant.

RESULTS

Effect of extraction mode on yield

The impact of extraction mode on extract yield of different parts of the plant is given in Table 1. The extract yield was found to be higher in Soxhlet extraction than cold maceration. However, among both modes of extraction, the yield of methanol extract was found to be higher than that of the hexane extracts. The lower yield of hexane extracts indicates that all the parts contain a lower amount of non-polar compounds.

Effect of extraction mode on constituents

The phytoconstituents of 4 parts of the plant extracts were affected by extraction mode, as shown in Table 2. These results indicated that methanol extract of all the parts exhibited higher contents of total polyphenols and tannins prepared by Soxhlet heat, whereas methanol extract of all parts prepared by maceration contained a higher amount of total flavonoid and triterpenoids.

Effect of extraction mode on antidiabetic activity

The methanol extracts of all parts prepared by maceration exhibited higher antidiabetic activity by all models than Soxhlet extracts. The macerated extracts of different parts were significantly different from standard (p<0.05) except flower and stem hexane extracts. The enzyme inhibition activity of extracts is given in Fig. 1A. The flower methanol extract was found to be a more active inhibitor than acarbose. The order of inhibitory potential of different extracts for alpha-amylase was flower> stem> leave> root. The dose-dependent response (50-1000 µg/mL) was used to calculate the IC₅₀ of the most active part extract (flower methanol extract). It was 600 µg/mL, as shown in Fig. 1B.

The glucose uptake enhanced by extracts of different parts is given in Fig. 2A. These results indicated that extracts had a higher ability to enhance glucose uptake. Glucose uptake was observed more in stem methanol extract than metformin. The dose-dependent response (50-1000 µg/mL) was used to calculate the EC₅₀ of the stem's most active part (methanol extract). It was 400 µg/mL, as shown in Fig. 2B.

At different time intervals, the non-enzymatic Hb-anti glycation potential of extracts and gallic acid results depicted that the potential of anti-glycosylation was maximum at 72 h and minimum at 24 h. The flower methanol extracts showed higher inhibition of glycosylation (70.11 ± 0.75%) as compared to standard at 72 h. At 72 h the order of anti-glycation potential was flower > stem > leave > latex > root extract. The result of these assays is shown in Fig. 3A-C. The IC₅₀ of most active flower methanol extract at 72 h was 420 µg/mL, as shown in Fig. 3D.

Table 1. Impact of extraction mode on yield of different extracts *Calotropis procera*.

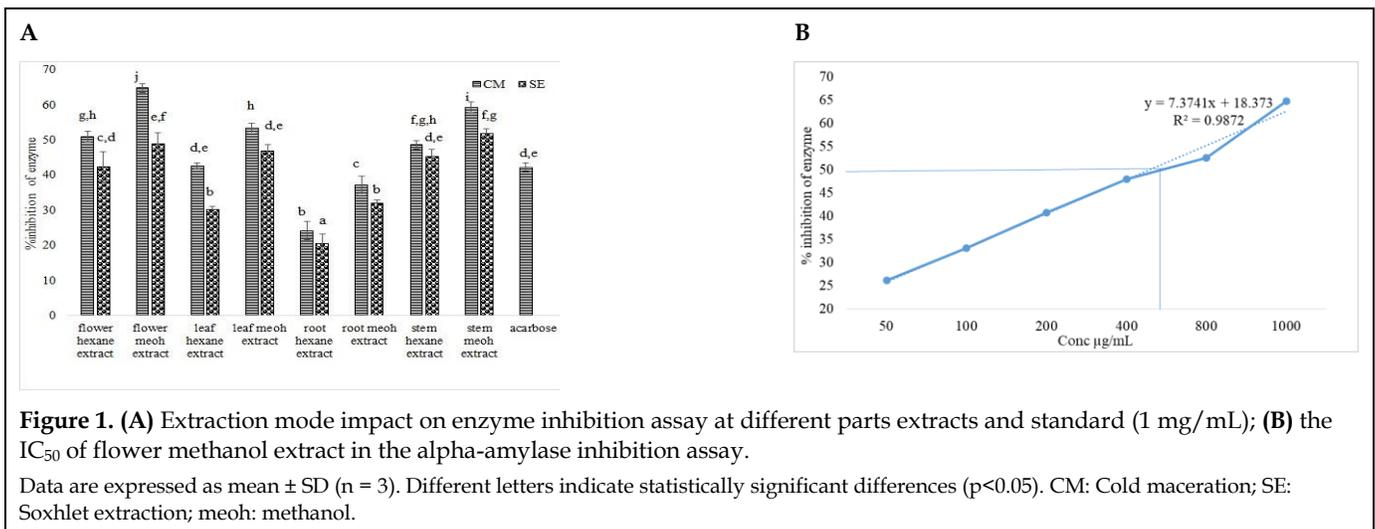
Extraction mode	Solvent	Yield (%)			
		Flower	Leaf	Root	Stem
Maceration	Hexane	7.8 ± 0.4 ^e	5.8 ± 0.45 ^e	9.8 ± 0.65 ^{de}	5.8 ± 0.30 ^e
	Methanol	34.3 ± 4.5 ^{bc}	35.3 ± 2.5 ^{bc}	36.0 ± 4.0 ^{bc}	38.8 ± 2.2 ^b
Soxhlet extraction	Hexane	9.7 ± 0.72 ^{de}	13.0 ± 2.0 ^d	14.2 ± 2.5 ^d	13.4 ± 1.5 ^d
	Methanol	43.8 ± 1.0 ^a	35.9 ± 1.8 ^{bc}	31.7 ± 2.1 ^c	38.4 ± 4.0 ^b

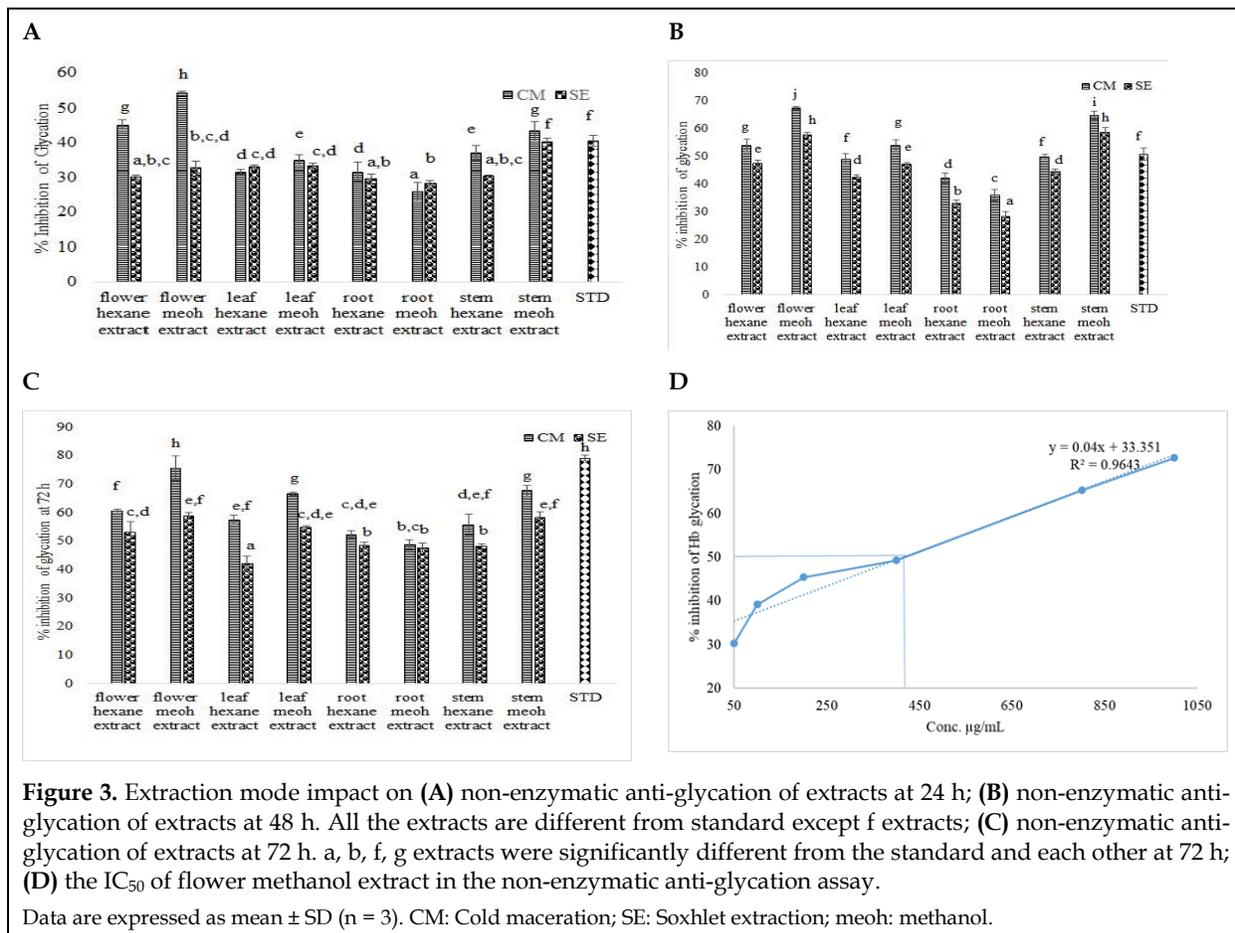
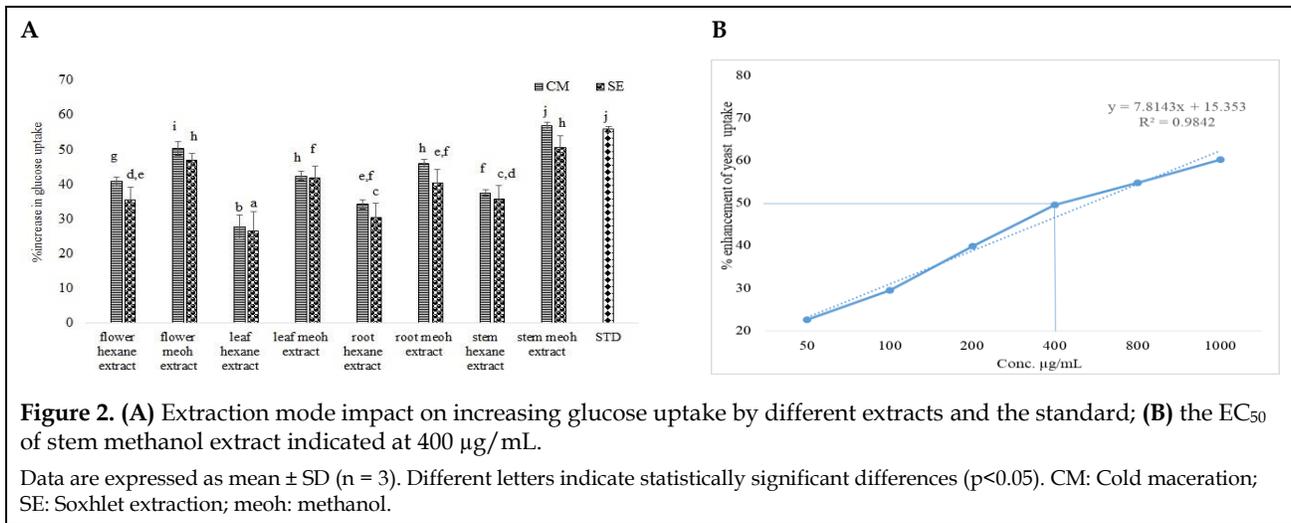
Data are expressed as mean ± SD (n = 3). Different letters indicate statistically significant differences (p<0.05).

Table 2. Extraction mode effect on phytochemical constituents of 4 parts extracts of *Calotropis procera*.

Extracts	Total polyphenols (mgGAE/g)		Total flavanoid (mgQE/g)		Total tannins (mgTAE/g)		Total triterpenoid (mgLE/g)	
	CM	SE	CM	SE	CM	SE	CM	SE
FLHE	39.4 ± 1.7 ⁿ	69.9 ± 2.6 ^k	295 ± 2.5 ⁱ	174.5 ± 2.5 ^k	17.5 ± 2.5 ^j	123.3 ± 3.8 ^d	97.7 ± 0.25 ^l	42.7 ± 2.7 ^o
FLME	393.5 ± 2.6 ^c	430.2 ± 0.99 ^a	685.8 ± 5.2 ^a	595.0 ± 2.5 ^c	169.1 ± 1.4 ^b	228.3 ± 3.8 ^a	330.0 ± 2.5 ^c	280.0 ± 2.5 ^e
LEHE	61.4 ± 1.8 ^l	199.2 ± 4.9 ^h	346.6 ± 1.4 ^h	299.0 ± 1.4 ⁱ	30.0 ± 2.5 ⁱ	57.5 ± 2.5 ^g	180.8 ± 3.8 ⁱ	121.6 ± 1.4 ^k
LEME	249.2 ± 1.9 ^g	369.9 ± 3.9 ^d	637.5 ± 2.5 ^b	556.6 ± 1.4 ^d	93.3 ± 1.4 ^f	120.0 ± 2.5 ^d	233.3 ± 5.2 ^g	221.6 ± 5.7 ^h
RTHE	7.8 ± 2.9 ^o	46.9 ± 4.9 ^m	282.7 ± 2.5 ^j	270.0 ± 2.5 ^k	45.0 ± 2.5 ^h	6.6 ± 2.8 ^k	63.3 ± 5.2 ⁿ	8.8 ± 1.2 ^p
RTME	255.0 ± 1.7 ^g	303.8 ± 4.3 ^f	599.1 ± 3.8 ^c	519.1 ± 1.4 ^f	89.1 ± 1.4 ^f	105.8 ± 1.4 ^e	313.3 ± 3.8 ^d	242.5 ± 2.5 ^f
STHE	76.8 ± 0.9 ^j	180.2 ± 1.9 ⁱ	377.5 ± 5.0 ^g	295.0 ± 2.5 ⁱ	6.6 ± 1.4 ^k	19.1 ± 1.4 ^j	137.5 ± 2.5 ⁱ	87.9 ± 8.3 ^m
STME	321 ± 2.6 ^e	407.2 ± 4.3 ^b	633.3 ± 2.8 ^b	545.0 ± 2.5 ^e	120.0 ± 4.3 ^d	150.8 ± 3.8 ^c	428.3 ± 1.4 ^a	339.1 ± 6.2 ^b

Data are expressed as mean ± SD (n = 3). Different letters indicate statistically significant differences (p<0.05). FLHE: Flower hexane extract; FLME: Flower methanol extract; LEHE: Leaf hexane extract; LEME: Leaf methanol extract; RTHE: Root hexane extract; RTME: Root methanol extract; STHE: Stem hexane extract; STME: Stem methanol extract; GAE: Gallic acid equivalent; QE: Quercetin equivalent; TAE: Tannic acid equivalent; LE: Lupeol equivalent; CM: Cold maceration; SE: Soxhlet extraction.





DISCUSSION

The maceration and Soxhlet method of extraction depicts the distinctive effects on the yield, nature, and

activity of the extracts. This could be due to temperature differences, heat transfer mechanism, solvent-powder interaction time, and constituent structure. In the maceration, heat is transported via conduction and

convection (Azwanida, 2015). The components are also released from the plant matrix due to a difference in an ionic solvent concentration gradient, which may be attributed to heat exchange and mass transfer in maceration. Heat provides the activation energy required to rupture plant tissue and liberate constituents in Soxhlet extraction, and heated solvent repeatedly came into contact with the crude powder, shifting the transfer equilibrium (Zaidi, 2013). The surface tension of powder particles has decreased as a result of the increase in temperature, which has improved the solubility and resulted, the yield of the compounds (Vergara-Salinas et al., 2012), as evident in Table 1.

The functionality of phytochemical compounds in extracts depends not only on the quantity but also on the bond interaction of these compounds with other molecules. The heat may also be responsible for the variation of the extract constituents. In plants, polyphenol oxidase enzyme, at ambient temperature causes the degradation of polyphenols in cells, but at high temperature, enzyme denatures and inhibits polyphenols degradation (Shaimaa and Mahmoud, 2016) and might cause dehydration of cell-matrix by breaking down polyphenol lipoprotein interactions, lowers the viscosity of the solvent to enter the plant matrix, promotes the diffusion coefficient and solubility rate of phenolic and tannin constituents in the solvent (Rajbhar et al., 2015; Jovanović et al., 2017). These factors might be responsible for higher polyphenol and tannin content in Soxhlet extraction extracts. This result is comparable with the previous study on *Diospyros melanoxylon* (Harun Al Rashid et al., 2019). The structure of thermosensitive constituents like flavonol, rutin found in *Calotropis procera*, degrades as the temperature rises. The presence of an oxygen moiety in a structure promotes deterioration, either direct oxidative mechanism or through the action of oxidizing enzymes, and makes the structure unstable (Ioannou et al., 2012). Because of this, total flavonoid and triterpenes levels may be higher in maceration extracts than Soxhlet extracts. The findings are comparable with a previous study (Okoduwa et al., 2016).

Some bioactive constituents in a plant are either heat sensitive or solvent specific. The selection of extraction mode has an impact on biological activity too. All the antidiabetic models showed that methanol extracts of all parts at ambient temperature exhibited potent antidiabetic activity, which may be attributed due to the presence of higher flavonoids and terpenoids contents (Hanhineva et al., 2010; Odjakova et al., 2012; Al-Ishaq et al., 2019). The polyphenolic and tannins may also

exhibit a synergic effect at ambient temperature. The luteolin, rutin, quercetin, chlorogenic acid, and ellagic acid, etc., are known for potent antidiabetic potential and, mentioned in plant literature too, may be responsible for this activity. In inhibitory enzyme assay, alpha-amylase is inhibited by plant extracts and acarbose, which make it unable to convert starch into glucose and ultimately lowers the glucose level (Tamil et al., 2010). In the glucose uptake model, the increased uptake of glucose from the reaction mixture to the yeast cell was observed. The glucose entered in a cell by facilitated diffusion as glucose concentration decreased within the cell due to more utilization of glucose (Teusink et al., 1998; Ahmed et al., 2009). The metformin and plant extracts have the capability to enhance the glucose uptake from the reaction mixture to yeast cells. In the non-enzymatic Hb anti-glycosylation model, protein and glucose binding resulted in glycosylated hemoglobin. Early glycosylation products called Amadori products, which resulted in advanced glycation products, which are stable and irreversible and cause diabetic complications. The binding of protein and glucose increased with the passage of time, and more glycosylated products formed. But the extracts' antioxidant potential inhibits their binding and the formation of glycosylated products at 72 h by trapping the reactive carbonyl intermediates in advanced glycated end products (James et al., 2011).

CONCLUSION

This research concludes that maceration and methanol have an effective capability to extract phytochemical constituents responsible for antidiabetic activity. The study also illustrates the various mechanism of action for managing diabetes through plant extracts. The study may be helpful for the isolation of antidiabetic compounds in the near future, as the most active part and extract constituents has been indicated in the present work.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

- Abubakar AR, Haque M (2020) Preparation of medicinal plants: Basic extraction and fractionation procedure for experimental purposes. *J Pharm Bioallied Sci* 12: 1-10.
- Adisa RA, Oke J, Olomu SA, Olorunsogo O (2004) Inhibition of human haemoglobin glycosylation by flavonoid containing leaf extract of *Cnestis ferruginea*. *J Cameroon Acad Sci* 4: 351-359.
- Ahmed F, Sudha S, Asna U (2009) Effect of various ayurvedic formulations and medicinal plants on carbohydrate hydrolyzing enzymes and glucose uptake by yeast cells - an *in vitro* study. *J Pharm Res* 2: 563-568.
- Alif AH, Hossin A, Hassaon MK, Madhu TM (2018) Phytochemical and pharmacological evaluation of *Cyperus odoratus* extracts. *Bangladesh Pharm J* 21:150-159.
- Al-Ishaq RK, Abotaleb M, Kubatka P, Kajo K, Büsselberg D (2019) Flavonoids and their antidiabetic effects: Cellular mechanisms and effects to improve blood sugar levels. *Biomolecules* 9: 430.
- Al-Taweel AM, Perveen S, Fawzy GA, Attiq Ur Rehman, Khan A, Mehmood R, Fadda LA (2017) Evaluation of antiulcer and cytotoxic potential of the leaf, flower, and fruit extracts of *Calotropis procera* and isolation of a new lignan glycoside. *Evid Based Compl Alt* 2017: 10.
- Azwani NN (2015) A Review on the extraction methods use in medicinal plants, principle, strength and limitation. *Med Aromat Plants* 4: 3.
- Bhaskar VH, Ajay SS (2009) Antihyperglycemic and antihyperlipidaemic activities of root extracts of *Calotropis procera* (Ait.) R.Br on streptozotocin induced diabetic rats. *Jordan J Biol Sci* 2: 177-180.
- Chang C, Yang M, Wen H, Chern J (2002) Estimation of total flavonoid content in propolis by two complementary colorimetric method. *J Food Drug Anal* 10:178-182.
- Cirillo VP (1962) Mechanism of glucose transport across the yeast cell membrane. *J Bacteriol* 84: 485-491.
- Fan JP, He CH (2006) Simultaneous quantification of three major bioactive triterpenes acids in the leaves of *Diospyros kaki* by high performance liquid chromatography method. *J Pharm Biomed Anal* 41: 950-956.
- Hanhineva K, Törrönen R, Bondia-Pons I, Pekkinen J, Kolehmainen M, Mykkänen H, Poutanen K (2010) Impact of dietary polyphenols on carbohydrate metabolism. *Int J Mol Sci* 11: 1365-402.
- Harun Al Rashid M, Majumder S, Mandal V, Mandal SC, Thandavarayan RA (2019) In search of suitable extraction technique for large scale commercial production of bioactive fraction for the treatment of diabetes: The case *Diospyros melanoxylon Roxb*. *J Tradit Complement Med* 9: 106-118.
- Ioannou I, Hafsa I, Hamdi S, Charbonnel C, Ghoul M (2012) Review of the effects of food processing and formulation on flavonol and anthocyanin behavior. *J Food Eng* 111: 208-217.
- Jalalpure SS, Salahuddin M, Imtiyaz Shaikh M, Manvi FV (2009) Anticonvulsant effects of *Calotropis procera* root in rats. *Pharm Biol* 1: 162-167.
- James SA, Auta R, Goje DJ (2011) *In vitro* study on inhibition of glycosylation of methanolic extract of *Hibiscus cannabinus*. *Sci World J* 6: 7-9.
- Jovanović A, Petrović P, Đorđević V, Zdunić G, Šavikin K, Bugarski B (2017) Polyphenols extraction from plant sources. *Lekovite Sirovine* 37: 37-42.
- Kamath JV, Rana AC (2002) Preliminary study on antifertility activity of *Calotropis procera* roots in female rats. *Fitoterapia* 73: 111-115.
- Kazeem MI, Mayaki AM, Ogungbe BF, Ojekale AB (2016) *In vitro* studies on *Calotropis procera* leaf extracts as inhibitors of key enzymes linked to diabetes mellitus. *Iran J Pharm Res* 15: 37-44.
- Larhsini M, Bousaid M, Lazrek HB, Jana M, Amarouch H (1997) Evaluation of antifungal and molluscicidal properties of extracts of *Calotropis procera*. *Fitoterapia* 68: 371-373.
- Magalhaes HIF, Ferreira PMP, Moura ES, Torres MR, Alves APNN, Pessoa ODL, Costa-Lotufo LV, Moraes MO, Pessoa C (2010) *In vitro* and *in vivo* antiproliferative activity of *Calotropis procera* stem extracts. *An Acad Bras Cienc* 82: 407-416.
- Neto MCL, de Vasconcelos CFB, Thijan VN, Caldas GFR, Araújo AV, Costa-Silva JH, Amorim ELC, Ferreira F, de Oliveira AFM, Wanderley AG (2013) Evaluation of anti hyperglycaemic activity of *Calotropis procera* leaves extract on streptozotocin-induced diabetes in Wistar rats. *Rev Bras Farmacogn* 23: 913-919.
- Odjakova M, Popova E, Merilin Al Sharif, Mironova R (2012) Plant derived agents with anti-glycation activity. *Intech Open. Glycosylation*. Petrescu S. Ed. DOI: 10.5772/48186.
- Okoduwa SIR, Umar AI, James DB, Inuwa HM, Habila JD (2016) Evaluation of extraction protocols for antidiabetic phytochemical substances from medicinal plants. *World J Diabetes* 7: 605-614.
- Parker KM, England JD, Da CJ, Hess RL and Goldstein DE (1981) Improved colorimetric assay for glycosylated hemoglobin. *Clin Chem* 27: 669-672.
- Rajbhar K, Dawda H, Mukundan U (2015) Polyphenols: Methods of extraction. *Sci Revs Chem Commun* 5: 1-6.
- Rohit S, Thakur GS, Sanodiya BS, Savita A, Pandey M, Sharma A, Bisen PS (2012) Therapeutic potential of *Calotropis procera*: A giant milkweed. *IOSR J Pharm Biol Sci* 4: 42-57.
- Saba AB (2011) Anti-inflammatory and analgesic activities of ethanolic leaf extract of *Calotropis procera*. *Afr J Biomed Res* 14: 203-208.
- Setty SR, Absar QA, Swamy Viswanath AHM, Tushar P, Prakash T, Prabhu K, Veeran G (2007) Hepatoprotective activity of *Calotropis procera* flowers against paracetamol-induced hepatic injury in rats. *Fitoterapia* 78: 451-454.

- Shaimaa GA, Mahmoud MS (2016) Effect of heat treatment on phenolic and flavonoid compounds and antioxidant activities of some Egyptian sweet and chilli pepper. Nat Prod Chem Res 4: 3.
- Singleton VL, Orthofer R, Lamuela-Raventós RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Meth Enzymol 299: 152-178.
- Sudesh G, Bhawana G, Karishma K, Pooja S (2012) Ethnopharmacological potential of *Calotropis procera*. An overview. Int Res J Pharm 3: 19-22.
- Tamil IG, Dineshkumar B, Nandhakumar M, Senthilkumar M, Mitra A (2010) *In vitro* study on α -amylase inhibitory activity of an Indian medicinal plant, *Phyllanthus amarus*. Indian J Pharmacol 42: 280-282.
- Teusink B, Diderich JA, Westerhoff HV, Van Dam K, Walsh MC (1998) Intracellular glucose concentration in derepressed yeast cells consuming glucose is high enough to reduce the glucose transport rate by 50%. J Bacteriol 180: 556-562.
- Tour N, Talele G (2011) Anti-inflammatory and gastromucosal protective effects of *Calotropis procera* (Asclepiadaceae) stem bark. J Nat Med 65: 598-605.
- Vergara-Salinas JR, Perez-Jimenez J, Torres JL, Agosin E, Perez-Correa JR (2012) Effects of temperature and time on polyphenolic content and antioxidant activity in the pressurized hot water extraction of deodorized thyme (*Thymus vulgaris*). J Agric Food Chem 60: 10920-10929.
- Zaidi BYM (2013) Study on factors affecting extraction of carotene from carrot by using Soxhlet extraction method. Ph.D Thesis, Universiti Malaysia Pahang, Malaysia.
- Zhang QW, Lin LG, Ye WC (2018) Techniques for extraction and isolation of natural products: a comprehensive review. Chin Med 13: 20.

AUTHOR CONTRIBUTION:

Contribution	Iftikhar S	Hussain K	Saleem M	Shehzadi N	Saghir F	Shaukat A	Naheed S	Siddique S
Concepts or ideas		x						
Design		x						
Definition of intellectual content		x						
Literature search	x							
Experimental studies	x			x				
Data acquisition	x			x	x	x		x
Data analysis	x	x						
Statistical analysis	x	x						
Manuscript preparation	x							
Manuscript editing	x	x						
Manuscript review	x	x	x	x	x	x	x	x

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