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In vitro pharmacological attributes and metabolite's fingerprinting of *Conocarpus lancifolius*

[Atributos farmacológicos *in vitro* y huellas dactilares de metabolitos de *Conocarpus lancifolius*]

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Abstract: Search for safe antioxidants and novel nutraceuticals urged to evaluate the antioxidant, anti-acetylcholine esterase and anti-lipoxygenase activity of various leaf extracts of *Conocarpus lancifolius*. Extraction was optimized from freeze dried plant extracts quenched with liquid nitrogen using water, ethanol, methanol, hexane, ethyl acetate and chloroform. Maximum extract yield, total phenolic contents and total flavonoid contents were obtained in case of ethanolic extraction. The highest 2,2-diphenyl-1-picrylhydrazylradical scavenging in terms of IC_{50} value of 55.26 µg/mL was observed for ethanolic leaf extract. The acetylcholine esterase and lipoxygenase inhibitory activities (IC_{50}) were also observed for ethanolic extract. These findings for ethanolic extract were statistically significant when compared with other extracts (ρ<0.05). The haemolytic % values indicated that all extracts were associated with very low or negligible toxicity. The epicatechin, isorhamnetin, rutin, scopoleptin, skimmianine, quercetin-3-O-α-rhamnoside, quercetin-3-O-β-glucoside, cornoside, creatinine, choline, pyruvic acid, α-hydroxybutyric acid, phyllanthin and hypophyllanthin were identified as major functional metabolites in ethanolic leaf extract of *C. lancifolius* by ^{1}H-NMR. The identified metabolites were probably responsible for the pharmacological properties of *C. lancifolius*. The findings may be utilized as pharmacological leads for drug development and food fortification.

Keywords: *Conocarpus lancifolius*; Antioxidant; Acetylcholine esterase; Lipoxygenase; ^{1}H-NMR analysis.

Resumen: Se insta a la búsqueda de antioxidantes seguros y nuevos nutracéuticos para evaluar la actividad antioxidante, anti-acetilcolina esterasa y anti-lipoxigenasa de varios extractos de hojas de *Conocarpus lancifolius*. La extracción se optimizó a partir de extractos de plantas liofilizados enfriados con nitrógeno líquido usando agua, etanol, metanol, hexano, acetato de etilo y cloroformo. En el caso de extracción etanólica se obtuvo el rendimiento máximo de extracto, el contenido de fenoles totales y el contenido de flavonoides totales. La mayor eliminación de radicales 2,2-difenil-1-picrilhidrazilo en términos de valor de CI_{50} de 55,26 µg/mL se observó para el extracto de hoja etanólico. También se observaron las actividades inhibidoras de la acetilcolina esterasa y lipoxigenasa (CI_{50}) para el extracto etanólico. Estos hallazgos para el extracto etanólico fueron estadísticamente significativos en comparación con otros extractos (ρ<0.05). Los valores del % hemolítico indicaron que todos los extractos estaban asociados con una toxicidad muy baja o insignificante. Se identificaron la epicatequina, isorhamnetina, rutina, escopoleptina, skimmianina, quercetina-3-O-α-ramnosido, quercetina-3-O-β-glucósido, cornosido, creatinina, colina, ácido pirúvico, ácido α-hidroxibutírico, filantrina e hipofillantina. como metabolitos funcionales principales en el extracto etanólico de hojas de *C. lancifolius* por 1H-NMR. Los metabolitos identificados probablemente fueron responsables de las propiedades farmacológicas de *C. lancifolius*. Los hallazgos pueden utilizarse como pistas farmacológicas para el desarrollo de fármacos y la fortificación de alimentos.

Palabras clave: *Conocarpus lancifolius*; Antioxidante; Acetilcolina esterasa; Lipoxigenasa; Análisis de ^{1}H-NMR.

INTRODUCTION

An important medicinal property of plants is their antioxidant activity due to presence of various bioactive ingredients. Plant based biologically active fractions and molecules have gained a tremendous importance due to their vital role as antioxidants, antimicrobial agents, pharmacological moieties and nutraceuticals (Singh *et al*., 2016). Antioxidants play a vital role to encounter the reactive oxygen species (ROS) to avoid the development of oxidative stress (Raza *et al*., 2018). The role of ROS is highly damaging and is associated with initiation and propagation of neurodegenerative disorders like Alzheimer's disease, Huntington's disease, Parkinson's disease and spinocerebellar ataxia (Liu *et al*., 2017).

It has been established that antioxidants play a pivotal role to scavenge the ROS at molecular levels to minimize the harmful health impacts associated with the phenomenon of oxidation. Plants serve as potential source of safe novel antioxidants and medicinally important substances even in presence of many organic drugs and synthetic antioxidants (Nadeem *et al*., 2019). Phenolic compounds are widely distributed in plants and serve as potential non-enzymatic defense system against the ROS and oxidative stress oriented neurodegenerative diseases (Pohl *et al*., 2018). Toxic behavior of synthetic antioxidants and medicines is an issue which strengthens the cause to search novel and natural sources of antioxidant and pharmacological entities. Toxicity of synthetic antioxidants and side effects of synthetic drugs also serve as an impetus to explore the plants as a complementary part of naturopathic approach. There are many questions on safety of commonly used synthetic antioxidants like butylated hyroxyanisole, butylated hydroxytoluene and ter-butyl hydroquinone (Yang *et al*., 2018). Natural antioxidants from plants are safe and more stable even at higher temperatures than synthetic antioxidants so may serve as an alternate to synthetic antioxidants (Taghvaei & Jafari, 2015).

Biologically active substances like phenolic compounds and flavonoids are also active to inhibit certain hazardous enzymatic actions. Therefore, plant extracts may act as effective agents against many health deteriorating enzymes like acetylcholine esterase (ACHe) and lipoxygenases (LOX), when in excess. Acetylcholine is a neurotransmitter in brain and body of humans and other animals for proper functioning of nervous system especially muscles. ACHe is the enzyme that hydrolyzes the acetylcholine and causes Alzheimer's disease. It has been established that ACHe inhibitors are very effective agents to alleviate the symptoms of Alzheimer's disease. Donepazil, tacrine and rivastigmine are licensed as ACHe inhibitors but these drugs are associated with gastrointestinal disorder, nausea, diarrhea, vomiting, muscle cramps and respiratory disorders (Tanaka *et al*., 2009; Sharma, 2019). Plant extracts are frequently investigated for their medicinal activities. The plants are well known for their therapeutic role including the treatment of neurodegerative disorders by inhibiting the ACHe and LOX. Metabolites from plant may serve as ACHe inhibitors to improve the concentration of neurotransmitter in brain (Attanayake & Jayatilaka, 2018). Lipoxygenases (LOXs) are associated with inflammatory diseases like, asthma, arthritis, skin allergies and neurodegenerative disorders. Many synthetic compounds are being used as anti-LOX inhibitors but again the toxicity of these substances motivates to discover new safe anti-LOX substances. Many plants have been studied for their biological activities but many are still present to explore.

Recently, *Cocnocarpus lancifolius* (*C. lancifolius*) of family Combretaceae has emerged as potential medicinal plant as indicated by previously performed studies. *C. lancifolius* has been reported to possess antibacterial (Ali *et al*., 2013), antioxidant, phytotoxic, and urease inhibitory properties (Saadullah *et al*., 2016). The *C. lancifolius* leaves are being consumed to treat various disorders like pneumonia, cough, diabetes and skin infections in local medicinal system of Pakistan. However, a little scientifically proved information is available on treatment efficacy and nature of metabolites present in *C. lancifolius* leaves. Moreover, this plant has never been explored for ACHe and LOX inhibitory properties. The current study was designed to investigate the antioxidant, anti-ACHe activity, anti-LOX activity, iron chelating potential and toxicity of various extracts of *C. lancifolius* leaves along with metabolite fingerprinting by ^{1}H-NMR.

MATERIALS AND METHODS

Chemicals and reagents

Folin-Ciocalteu reagent (FC), Butylated hydroxyltoluene (BHT), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 5,5´-dithiobis [2-nitrobenzoic acid] (DTNB), Aetylcholine iodide, gallic acid from Sigma-Aldrich (USA) were used.

Acetylcholine esterase was arranged from Centre of Excellence in Molecular Biology Lahore, Pakistan. All the solvents and miscellaneous chemicals were of analytical grade.

Plant material collection and identification

The fresh leaves of *C. lancifolius* were harvested and identified form the Department of Botany, GC University Lahore, Pakistan.

Extraction process

The leaves were quenched immediately with liquid nitrogen to stop all the metabolic processes. After grinding, powdered samples were subjected to freeze drying for 48 Hrs. The powdered samples were added in water, ethtyl acetate, methanol, ethanol, chloroform or hexane in 1:10, separately. Pure solvents were used for extraction. The samples were shaken for 24 hours. After that samples were sonicated for 30 min on soniprep 150 Sanyo instrument, UK. Samples were covered with aluminium foil to avoid photo-degradation of metabolites. The samples were filtered on suction assembly and excess solvent was evaporated under vacuum on rotary evaporator. The waxy material was again free dried at -68°C until complete dryness and stored at -81°C till further use.

Total phenolic contents (TPC)

TPC were determined by following the established method with little modification (Zengin *et al*., 2010). Initially, 20 µL of each plant extract was added the 90 µL of Folin-Ciocalteu reagent followed by addition of 91 µL of 10% Na_2CO_3. Absorbance was noted on 726 nm on spectrophotometer. Results were reported as Gallic acid equivalent mg/g dried extract (mg GAE/g D.E).

Total flavonoid contents (TFC)

For determination of total flavonoid contents (TFC), plant extracts (0.1 mg) in 2 mL of methanol were diluted with distilled water. Then of 0.5 mL of 5% solution of $NaNO_2$ was added to this mixture followed by addition of 10% $AlCl_3$. Incubation for 10 min was carried out followed by addition of 1M solution of NaOH was added and shaked to note absorbance at 510 nm on spectrophotometer (Zhishen *et al*., 1999). Values of TFC were presented in quercetin equivalent mg/g dried extract (QE mg/DE).

DPPH assay for antioxidant activity

Free radical scavenging activity of plant extracts was determined using DPPH assay (Fki *et al*., 2005). A concentration of DPPH in methanol was made by dissolving 2.5 g of DPPH in methanol. Plant extracts were dissolved in methanol (1mg/mL) and 200 µL of each extract was added to 1.0 mL of DPPH solution. Reaction mixture was stored in dark for at least 30 mints and absorbance was measured at 517 nm on spectrophotometer. Free radical scavenging was calculated by using the following formula.

$$\%\ \text{DPPH inhibition} = \frac{A - B}{A} \times 100$$

Where A was absorbance of blank and B was absorbance of Sample. Quercetin was used as standard reference and results were represented as µg/mL. The IC_{50} values were computed by considering the % scavenging against extract concentration.

In vitro ACHe inhibition assay

Reaction mixture having 100 µL of acetylcholine esterase, 2.0 mL of 100 mMtris buffer with pH 7.8, 200 µL of each extract fractions and 100 µL DTNB was incubated for 15 min at 25°C. Then 200 µL of acetylcholine was added to start the reaction and stayed further for 30 mints till absorbance was taken at 412 nm (Shahwar *et al*., 2011). Following formula was used to find out the % inhibition.

$$\%\ \text{inhibition} = \frac{W - S}{W} \times 100$$

Where W represented enzymatic activity without test sample and S represented enzymatic activity with sample Galantamine (GL) from Merckwas used as standard reference drug.

***In vitro*lipoxygenase (LOX) inhibitory assay**

Spectrophotometric determination of anti-LOX activity was carried out by mixing 0.1 M phosphate buffer and test sample followed by 10 mints incubation at 25°C. After the addition of substrate, reading was noted at 234 nm using baicalein (Tocris Bioscience) as standard (Yawer *et al*., 2007). The percent LOX inhibition was calculated using the following relationship;

$$\%\ \text{inhibition} = (1 - A/B) \times 100$$

Where A is the enzymatic activity without any inhibitor and B is the enzyme activity in presence of inhibitor.

Iron chelating activity

Iron chelating activity was carried out for all extracts by using an already reported scheme (Dinis *et al.*, 1994). Briefly, 100 µL $FeSO_4$ (2 mM) were mixed with 0.2 mL of 5 mM ferrozine. Then 2 mg/mL of plant extract was mixed with the prepared reaction mixture. The mixture stayed at ambient conditions of temperature for 15 min followed by absorbance measurement at 562 nm. The EDTA was used as positive control. Iron chelating activity was calculated by following equations:

$$\text{Iron chelating activity} = (\text{Ac} - \text{As})/\text{Ac} \times 100$$

The control was also run which did not contain plant extract and its absorbance was represented by Ac and absorbance of sample was reported as As.

Toxicity evaluation

The cytotoxicity evaluation of extracts was determined by hemolytic assay as per reported method (Kumar *et al.*, 2011). Simply, 1000 µg/mL of each extract was added to NaCl (0.1%) and 2% human erythrocyte suspension. The mixture was incubated for 30 min and centrifugation was done. The supernatant was collected and absorbance at 540 nm was observed. Positive control (0.1% Triton) and negative control (phosphate buffer saline) were used. The measurements were made in triplicate and hemolytic % were calculated by following formula:

$$\text{Hemolytic \%} = \frac{Abs\ of\ sample - Abs\ of\ negative\ control}{Abs\ of\ positive\ control} \times 100$$

Metabolites' identification by ^{1}H-NMR

The major classes of primary and secondary metabolites were confirmed by using ^{1}H-NMR. The highly efficient crude extract was added in CH_3OH-d_4 and KH_2PO_4 buffer of pH 6.0 in D_2O having 0.1%. Sample was vortex for 2 min and ultrasonicated for 30 minutes at 30ºC. The sample was centrifuged at 13000 rpm for 10 minutes. Then 600 µL of supernatant was collected for ^{1}HNMR analysis on INOVA 500 MHz spectrometer (Varian Inc, CA), at 499.887 MHz frequency with tetramethylsilane (TMS) as internal standard (Mediani *et al.*, 2012a). The spectrum was bucketed with Mest Renova 11.0.

Statistical analysis

The results were evaluated for the statistical significance by using Minitab 17.0 software. The one way ANOVA test was applied to see the differences in means. The standard deviation (±) was also applied for triplicate values.

Table No. 1
Extract yields (%), TPC and TFC for *H. indica*

Solvent	Extract yield (%)	TPC mg GAE/g D.E	TFC mg QE/g D.E
Water	10.11 ± 0.02^{f}	149.67 ± 1.03^{e}	89.55 ± 1.03^{e}
Methanol	20.92 ± 0.03^{b}	236.82 ± 1.12^{b}	136.10 ± 1.12^{b}
Ethyl acetate	15.12 ± 0.02^{e}	158.64 ± 1.10^{d}	78.15 ± 1.10^{d}
Ethanol	22.09 ± 0.02^{a}	248.77 ± 1.11^{a}	148.92 ± 1.52^{a}
Chloroform	17.15 ± 0.02^{c}	180.80 ± 1.95^{c}	94.30 ± 1.95^{bc}
Hexane	16.01 ± 0.01^{cd}	180.02 ± 1.38^{c}	98.72 ± 1.38^{b}

RESULTS

Extract Yield (%), TPC and TFC

The extract yields, TPC and TFC obtained through different solvents are given in Table No. 1. The highest extract yield of 22.09 ± 0.02% was obtained with ethanol which was significantly higher than the yields given by other solvents (ρ<0.05). The highest TPC value of 248.77 ± 1.11 mg GAE/g D.E was observed in ethanolic extract and significantly higher than the TPC obtained through other solvent systems. The TFC of 148.92 ± 1.11 mg QE/g D.E were the maximum among all extracts. The aqueous extract

yielded lowest amounts of extracts and TPC while ethyl acetate produced lowest amount of TFC.

DPPH radical scavenging

The results of DPPH assay as IC_{50} value are given in Figure No. 1. The IC_{50} value (55.26 µg/mL) regarding DPPH radical scavenging for ethanolic extract was the lowest which indicated the significant antioxidant activity (ρ<0.05) among all extracts. The second highest antioxidant activity was observed for methanolic extract.

In-vitro anti-ACHe activity

The findings for ACHe inhibition are given as Figure No. 2. The results indicated that *C. lancifolius* leaf extract exhibited substantial ACHe inhibition activity. The ethanolic extract exhibited highest enzyme inhibition with IC_{50} value of 65.05 µg/mL, which was significantly higher when compared with other under study extracts (ρ<0.05).

In-vitro anti- LOX inhibitory activity

The experimental findings for LOX inhibition by *C. lancifolius* extracts are given in Figure No 3. Observations indicated that ethanolic extract exhibited substantial potential to inhibit LOX and the values were statistically significant as shown by ρ<0.05.

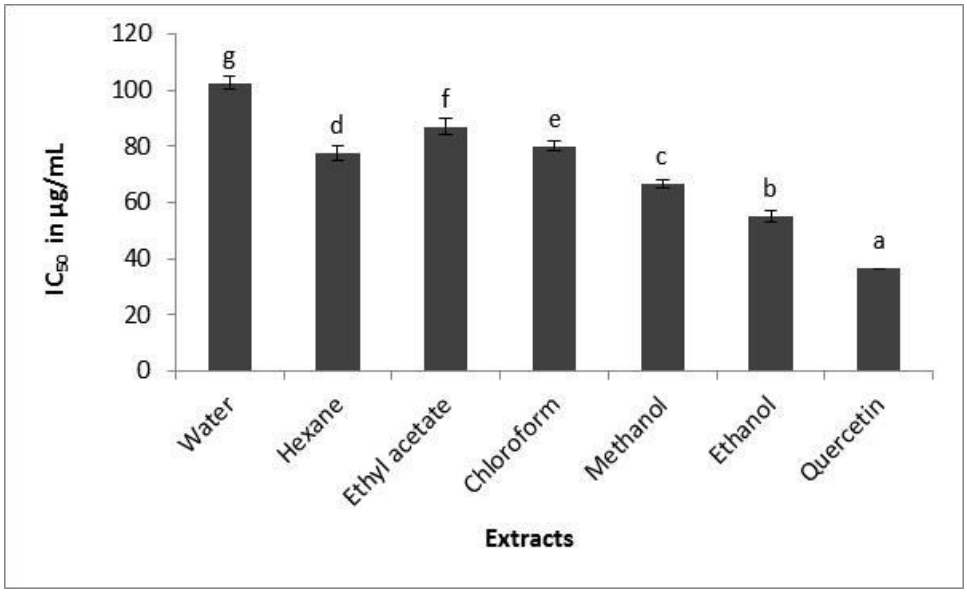

Figure No. 1
DPPH radical scavenging activity demonstrating the statistical significanceof *C. lancifolius* extracts

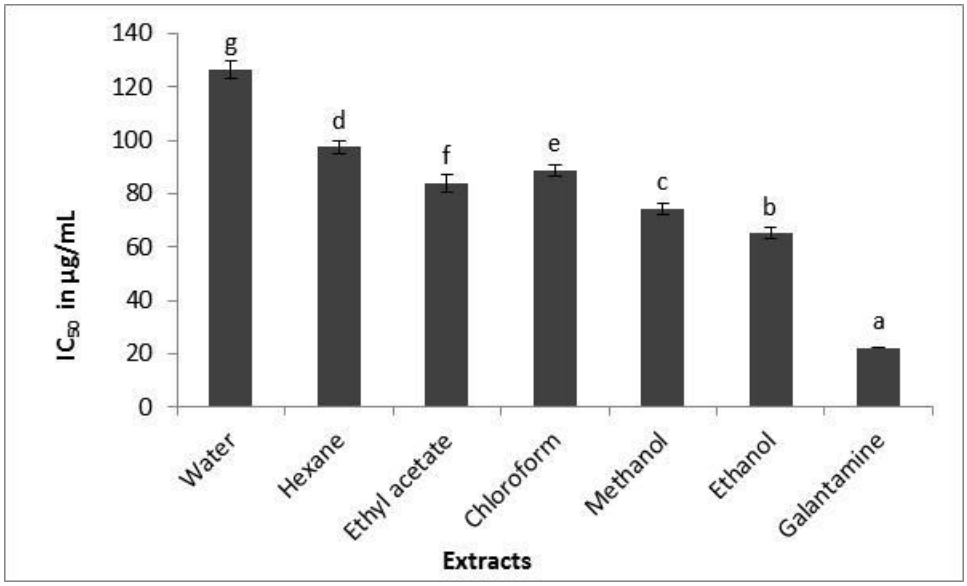

Figure No.2
ACHe inhibitory activity demonstrating the statistical significanceof *C. lancifolius* extracts

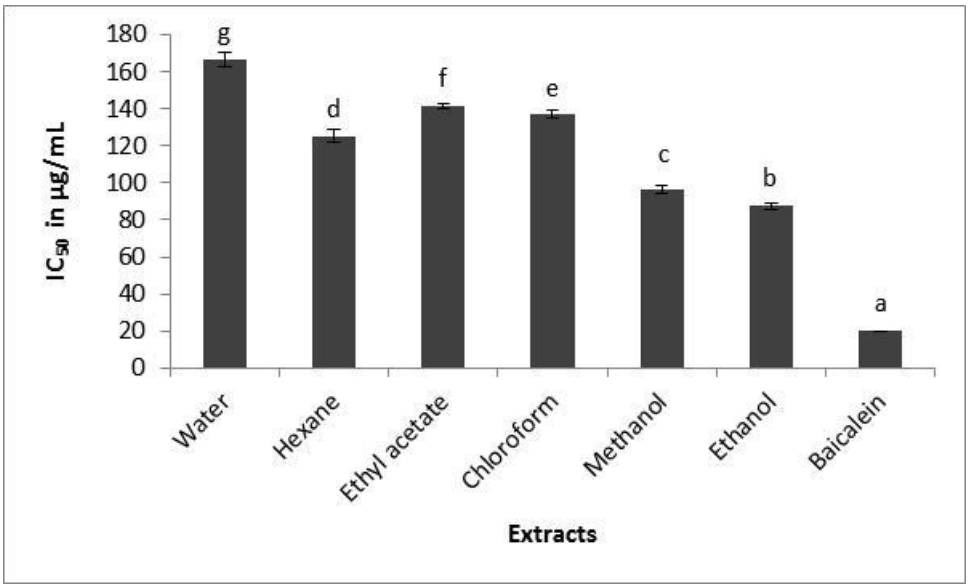

Figure No. 3
LOX inhibitory activity demonstrating the statistical significanceof *C. lancifolius* extracts

Iron chelating activity

The results of iron chelating assay are presented as Figure No. 4. The results were represented as % iron chelating activity. The findings explored that both methanolic and ethanolic extracts were almost of similar potential in this regard. The statistical analysis indicated that iron chelating % values for methanolicand ethanolic extracts was non-significant (ρ>0.05) but significantly higher than remaining extracts (ρ<0.05).

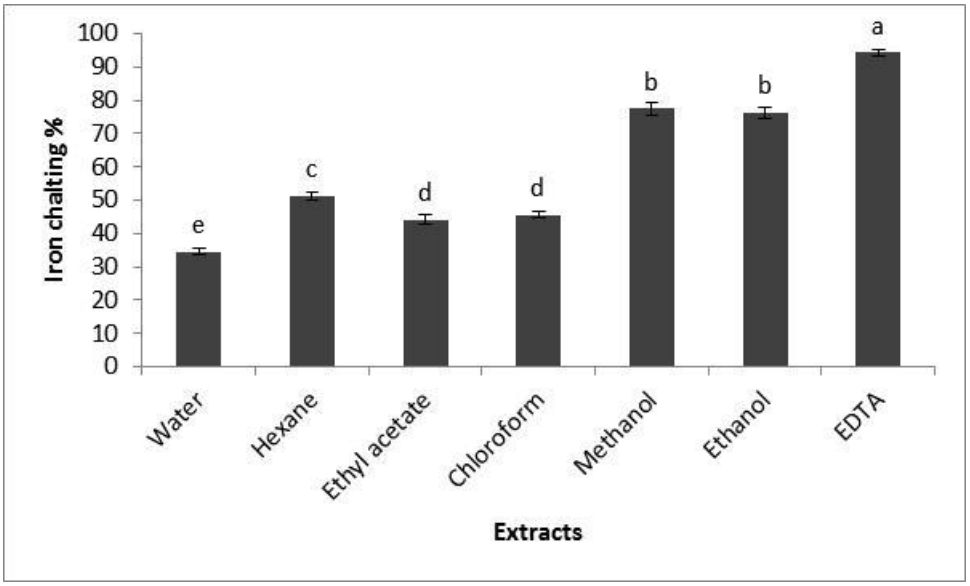

Figure No. 4
Iron chelating activity of demonstrating the statistical significance of *C. lancifolius* extracts

Toxicity evaluation by haemolytic assay

The Figure No. 5 represents the haemolytic % for each extract to evaluate the toxicity. All the extracts exhibited very low or negligible toxicity when compared with triton x-100.

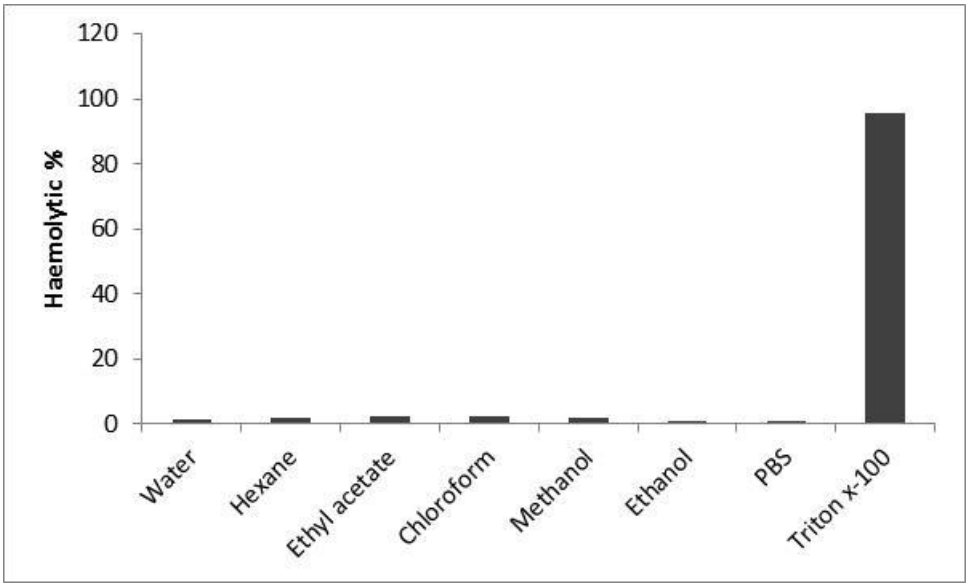

Figure No. 5
Haemolytic % of *C. lancifolius* extracts for toxicity evaluation

Metabolite identificationby ^{1}H-NMR

The ^{1}H NMR spectrum of ethanolic extract of *C. lancifolius*is given as Figure No. 6. The different regions corresponding to different classes of metabolites have been highlighted in figure. These regions were further explored for the identification of possible metabolites.

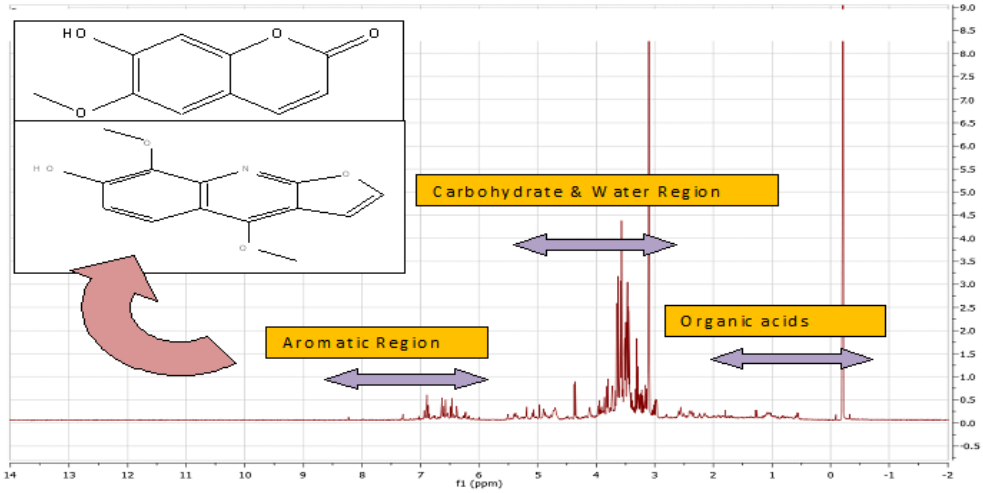

Figure No. 6
The ^{1}H NMR spectrum of ethanolic extract of *C. lancifolius*

The details of identified metabolites along with specific chemical shift values are presented in Table No. 2. The NMR spectrum elaborated the presence flavonoids (epicatechinδ = 7.03 (s), δ = 6.94 (s)), isorhamnetin δ = 3.14 (s), rutin (δ = 6.24 (d, *J* = 5.0 Hz), δ = 4.97 (d, *J* = 3.5 Hz)) alkaloids (scopoleptin δ = 6.89 (s), δ = 6.94 (s), δ = 3.82 (s), skimmianine δ = 4.11 (s), δ = 4.06 (s)), flavonoid derivatives (quercetin-3-O-α-rhamnoside δ = 6.27 (d, *J* = 7.5 Hz), quercetin-3-O-β-glucoside δ = 6.24 (d, *J* = 5.0 Hz), δ = 5.08 (d, *J* = 9.0 Hz), flavonoid glycoside (cornoside δ = 4.37 (d, *J* = 7.5 Hz)),organic acids (fumaric acid δ = 6.54 (s), pyruvic acid δ= 2.35 (s), 3-hydroxybutyric acid δ = 1.27 (d, *J* = 5.5 Hz)) and lignans (hypophyllanthin δ= 3.31 (s), phyllanthin δ = 3.33 (s)) in ethanolic leaf extract (Table No. 2). Flavonoids and their derivatives were associated with the chemical shift values in aromatic region (Mediani *et al*., 2012a; Mediani *et al*., 2012b; Al-Zuaidy *et al*., 2016). The confirmation of mentioned secondary metabolites of nutraceutical importance complemented the strong antioxidant and antidiabetic potency of *C. lancifolius* leaves. The 16 metabolites were profiled in the 60% ethanolic leaf extract of *C. lancifolius* and have never been reported for this plant previously. A previous report on *C. lancifolius* reported the absence of alkaloids in leaves (Saadullah *et al*., 2014) but the findings of current work revealed the presence of two important alkaloids, the scopoleptin and skimmianine.

Table. No. 2
Metabolites and their relative chemical shift (δ) values by ^{1}H NMR

Metabolite	^{1}H-NMR Characteristics
Epicatechin	δ = 7.03 (s), δ = 6.94 (s)
Scopoleptin	δ = 6.89 (s), δ = 6.94 (s), δ = 3.82 (s)
Isorhamnetin	δ = 3.14 (s)
Quercetin-3-O-α-rhamnoside	δ = 6.27 (d, *J* = 7.5 Hz)
Quercetin-3-O-β-glucoside	δ = 6.24 (d, *J* = 5.0 Hz), δ = 5.08 (d, *J* = 9.0 Hz)
Rutin	δ = 6.24 (d, *J* = 5.0 Hz), δ = 4.97 (d, *J* = 3.5 Hz)
Cornoside	δ = 4.37 (d, *J* = 7.5 Hz)
Skimmianine	δ = 4.11 (s), δ = 4.06 (s)
Fumaric acid	δ = 6.54 (s)
α-glucose	δ = 5.19 (d, *J* = 4.0 Hz)
Creatinine	δ = 3.94 (s), δ = 3.04 (s)
Choline	δ = 3.51 (m), δ = 3.22 (s), δ = 3.02 (s)
Pyruvic acid	δ= 2.35 (s)
3-Hydroxybutyric acid	δ = 1.27 (d, *J* = 5.5 Hz)
Hypophyllanthin	δ= 3.31 (s)
Phyllanthin	δ= 3.33 (s)

DISCUSSION

The plants are rich source of natural antioxidants and therapeutic agents to treat chronic ailments through oxidative stress oriented modulations. The polyphenols are the leading component of plants to impart antioxidant and medicinal properties and their determination in particular extract is an essential feature of studies on medicinal plants (William *et al*., 2018). The TPC and TFC analysis of *C. lancifolius* extracts revealed that ethanolic fraction exhibited highest amounts of polyphenols. The variation in polyphenol contents with respect to specific solvent was most probably due to the polarity of particular solventused for extraction. The solvent polarity of ethanol played vital role to bring the maximum phytochemicals in extract. A study on closely related specie, the *Conocarpus erectus* revealed that high extract yields were obtained with 60% ethanol and the same extract possessed high DPPH scavenging activity (Raza *et al*., 2018). The high quantities of phenolic and flavonoid contents were reflected to play their part in biological activities like DPPH scavenging (Mahmood *et al.,* 2020), ACHe and LOX inhibitions. The ethanolic extract was emerged as most potent fraction regarding antioxidant activity and enzyme inhibition properties. The stabilization of DPPH radical through proton donation by polyphenolic compounds was the most probable justification behind the antioxidant activity. The inhibition of ACHe and LOX by extracts of *C. lacifolius* leaves might be due to binding of functional groups of secondary metabolites with the active sites of enzymes. These site specific interactions are often observed when the secondary metabolites of plants come in contact with important amino acid residues of enzymes (Zhang *et al*., 2018). The ACHe inhibitors reduce the acetylcholine hydrolysis to maintain or increase the acetylcholine level in brain to smooth the neurotransmission for proper body functioning. This phenomenon is helpful to control the neural deficits which, consequently reduces the symptoms of neurodegenerative disorders (Ferreira *et al*., 2006). The ethanolic extract also inhibited the LOX activity most probably due to high TPC and TFC. The LOX enzyme adds molecular oxygen to unsaturated sites which reults in formation of hydroperoxides leading to inflammations (Ben-Nasr, 2015). The ethanolic extract of *C. lancifolius* showed promising LOX inhibition which confirmed the anti-inflammatory properties to *C. lancifolius*, most probably due to secondary metabolites. The iron chelating activity indicated that both, ethanolic and methanolic extracts showed similar potential and statistical analysis confirmed that there was no significant difference between both (ρ>0.05). The iron chelating activity is a good strategy to avoid ROS generation and extracts having high iron chelating activity are strong radical scavengers. The statistical analysis revealed that the finding of DPPH, ACHe and LOX inhibitory assays for ethanolic extract of *C. lancifolius* were significantly higher when compared with other extracts (ρ<0.05).

The aspect of metabolite's identification through reliable technique is an essential feature to find out the most probable reason behind the pharmacological potential of a particular plant extract. The ^{1}H-NMR analysis of ethanolic extract (most potent) revealed the presence of some high value secondary metabolites including epicatechin, isorhamnetin, rutin, scopoleptin, skimmianine, quercetin-3-O-α-rhamnoside, quercetin-3-O-β-glucoside and cornoside. The pharmacological significance of these identified phytoconsitituents cannot be ignored as these may be responsible for excellent antioxidant, ACHeand LOX inhibitory potential of *C. lancifolius*. The choline was also identified in ethanolic extract and it was reported that this micronutrient is involved in neuro-protection by maintaining the brain's health, structure of brain cells and signal regulation (Bekdash, 2019). The plants having strong antioxidant activities were reported to contain phytochemical including rutin, epicatechin, isorhamnetin, skimmianine and quercetin (Al-Zuaidy *et al*., 2016; William *et al.,* 2018; William *et al.,* 2019). A pharmacokinetic study revealed the brain penetration efficacy of epicathecin and catechin and proved their neuro-protective role (Wu *et al.,* 2012). Scopoleptin isolated from *Canarium patentinervium* Miq exhibited good ACHe inhibitory action with IC_{50} value of 51 µg/mL (Mogana *et al.,* 2014). An investigation reported that 80% methanolic extract of *Myricaria elegans* inhibited the ACHe activity but did not show anti-LOX activity (Ahmad *et al.,* 2003). Rutin is pharmacologically important flavonoid. A study on *Musa acuminate* indicated effective ACHe activity and phytochemical investigation showed rutin and kaempferol-3-O-rutinisode as major components of extract (Oresanya *et al.,* 2020). Quercetin was also reported as essential component of plant extract having antioxidant and LOX inhibitory properties (Paun *et al.,* 2018). Hence, the identified compounds may be of immense medicinal potential potential to treat oxidative stress oriented neurodegenerative disorders.

The toxicity of pharmacologically active extracts and compounds is highly demanded which enhances their acceptance among consumers. Thehaemolytic assay was conducted to evaluate the toxicity of all the extracts. The haemolytic % values demonstrated that all the extracts were associated with very low or negligible toxicity. The safety of extracts added a remarkable feature for further extension of study. The leaves of *C. lancifolius* can be further processed for *in-vivo* trials for development of a naturopathic approach in future towards neurodegenerative disorders and functional foods with antioxidant and pharmacological attributes.

CONCLUSION

The findings of current study provided valuable information on the pharmacological activities of *C. lancifolius*leaf extracts. The ethanol was proved as most appropriate choice to extract pharmacologically important compounds from leaves of *C. lancifolius*. The excellent antioxidant, ACHe and LOX inhibitory properties of ethanolic leaf extract were most probably due to synergistic impact of the identified metabolites. After careful *in-vivo* trials, the *C. lancifolius* leaves may be utilized as a rich source of safe, capacitive and functional metabolites to treat oxidative stress based neurodegenerative deficits and to add medicinal functionalities in food.

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