Chandana Choudhury Barua. *et al.* / *International Journal of Phytopharmacology.* 5(4), 2014, 252-260.

e- ISSN 0975 – 9328
Print ISSN 2229 – 7472

International Journal of Phytopharmacology

Journal homepage: www.onlineijp.com

IJP

CLERODENDRON INDICUM: A REPERTOIRE OF PHYTOCHEMICALS AND ITS ANTIOXIDANT ACTIVITY

***Chandana Choudhury Barua[1], Anindhya Sundar Das[1], Suparna Sen[1], Anindita Talukdar[1], Achinta Gohain Barua[2], Gakul Baishya[3], Subhan Chandra Nath[3]**

[1]Department of Pharmacology & Toxicology, [2]Veterinary Public Health, College of Veterinary Science, Khanapara, Guwahati 781022, Assam, India.
[3]Division of Natural Product Chemistry, Division of Medicinal, Aromatic & Economic Plants, CSIR-NEIST, Jorhat, Assam, India.

ABSTRACT

Clerodendron indicum, a widely distributed plant in South East Asia, has been reported for its various therapeutic properties. The aim of our study was to determine the phytochemicals preset there in both qualitatively and quantitatively and their antioxidant potentials. The ethanolic extract (EE) and hydroethanolic extract (HE) of *C. indicum* have been studied by preliminary phytochemical analysis, HPTLC and HPLC system. Assessment of antioxidant properties was done by hydroxyl radical scavenging assay, DPPH radical scavenging assay, super oxide radical scavenging assay and reductive ability assay using standard methods. HPTLC and HPLC study of the both the extracts showed the presence of quercetin. However quercetin of EE was higher than HE. Likewise, antioxidant activity of the HE extract was also inferior to EE. EE extract conferred significant antioxidant activity and higher flavonoid content than HE.

Key words: total phenolic content, total flavonoid content, antioxidants, HPTLC, HPLC, hydroxyl, DPPH, superoxide, reductive ability.

INTRODUCTION

In recent years, there is an upsurge in finding of newer antioxidants to scavenge its foes viz. Reactive Oxygen Species (ROS) (Kunwar *et al*., 2011) and Reactive Nitrogen Species (RNS) (Naskar *et al*., 2011). ROS and RNS are cumulative terms used for groups of oxidants which are either free radicals or molecular species capable to engender free radicals. Although ROS and RNS are essential for the normal physiological functions like gene expression, cellular growth, defense against infections (Droge *et al*., 2002), cellular signalling (Chiurchiu *et al*., 2011), they have detrimental effects in our body like lipid peroxidation leading to biological membrane damage (Devasagayam *et al*., 2003), DNA damage (Dizdaroglu *et al*., 1993; Pacher *et al*., 2007), protein damage associated with Alzheimer's disease, Parkinson's Disease (Butterfield, 1996 ; Winyard *et al*., 1998) etc. Carbohydrates are also affected by the OH• and produce carbon-centered radical which leads to chain breaks in important molecules like hyaluronic acid. In the synovial fluid of joints, neutrophils are congregated during inflammation and generate significant amounts of oxy-radicals, that is also being implicated in rheumatoid arthritis (Geldeman *et al*., 2007). Antioxidants are compounds which protect biomolecules from the damaging effects of ROS and RNS (Kunwar *et al*., 2011; Slemmer *et al*., 2008). Although, cells are outfitted with an impressive repertoire of antioxidant enzymes and small antioxidant molecules, these molecules may not be sufficient enough to normalize the redox status during oxidative stress (Geldeman *et al*., 2007). Under such conditions supplementation with exogenous antioxidants is required to restore the redox homeostasis in cells. Currently available synthetic antioxidant drugs have potential adverse affect on human health like carcinogenesis, cytotoxicity to name a few. These adverse

Corresponding Author

Chandana Choudhury Barua
Email: chanacin@gmail.com

affects can be ameliorated to a greater extent through natural plant based antioxidants (Chen *et al*., 1992). To employ plant based antioxidants for the benefit of human health, research has put forward steps to explore the novel antioxidant sources with greater significance (Seifried *et al*., 2007).

The genus *Clerodendrum* L. [Family Lamiaceae (Verbenaceae)] is very widely distributed in tropical and subtropical regions of the world and five hundred and eighty species of the genus have been identified till date (Shrivastava *et al*., 2007). Amongst them, *Clerodendrum indicum* (L.) Kuntze alias as turk's turbin, distributed in South East Asia, India, Nepal, Bhutan, Sri Lanka and Southern China (Manandhar *et al*., 2002). It is prevalent in Assam and locally nom de plume as "*Akolbih*". It is a perennial bushy plant that attains a height of 1-4 meters, stems are hollow; leaves in whorls of 3-6, 10-20 cm long and 2 to 8 cm in breadth with entire or serrated margins; inflorescences are large, about 60 cm in length and bear tubular white or bluish showy flowers; fruits are circular drupes with 4 drupelets. *C. indicum* blossoms and gives fruit in summers and rainy seasons.

The plant extracts of *C. indicum* have been traditionally used in scrofulos infection, buboes problem, venereal infection and skin diseases. Moreover, it has been used as a vermifuge and febrifuge (Rahman *et al*., 2000). Inflammatory diseases like rheumatism and asthma can also be treated with the root and leaf extracts of *C. indicum* (Seifried *et al*., 2007). Moreover, antinociceptive and antimicrobial activities of leaf extracts has been reported (Pal *et al*., 2012). The aqueous and ethanol extracts of plant of *C. indicum* showed the anti HIV (Bunluepuech *et al*., 2009). Root of the *C. indicum* reportedly contains two flavonoidic compounds such as pectolinarigenin and hispidulin and it has been reported to show significant antimicrobial activities against twelve pathogenic bacteria (Raihan *et al*., 2012). The juice of the fresh leaves has been prescribed by the folk practitioner as blood purifier, to treat gastrointestinal tract aliments (Pal *et al*., 2012).

Based on the previous reports on the therapeutic properties of *C. indicum*, our present study was aimed to screen the phytochemical content and the antioxidant activity of *C. indicum* using different *in vitro* models. The study was conducted with ethanolic (EE) and hydroethanolic (HE) extracts of *C. indicum*.

MATERIAL AND METHODS

In our study, the plant extracts of *C. indicum* were prepared wielding organic solvents viz. ethanol and hydroethanol for qualitative and quantitative phytochemical screening. Antioxidant activities of ethanolic and hydroethanolic extracts of the plant were assessed via Hydroxyl Radical Scavenging Assay; 2, 2-diphenyl-1-picrylhydrazyl Assay (DPPH); Superoxide Radical Scavenging Assay and Reductive Ability. A comparative study of antioxidant properties of different extracts of *C. indicum* has been done to correlate their antioxidant activities.

Plant Material

The ethanolic and hydroethanolic extracts of the *C.indicum* were obtained in the month of January-February 2012, from CSIR-NEIST, Jorhat, Assam. Voucher specimens (by I.C.Barua, 4915 & 4922) were prepared by following the guidelines of Botanical Survey of India, poisoned with mercuric chloride and processed to deposit in the Central National Herbarium (CAL), Howrah, and the Kanjilal Herbarium (ASSAM), Shillong. Meghalaya.

Extract Preparation

One kilogram of each dry plant material was dipped into 3 litres of ethanol and hydroethanol (ethanol: water , 50:50) separately in 5 litre round bottom flasks and the mixtures were mechanically stirred at room temperature for 7 days. The solid residues were filtered and the filtrates were concentrated under vacuum at 50^{o} C using rotary evaporator (Rotavapor R-210, Buchi) which gave 14.20 g of crude ethanol extract (yield 1.42%) and 13.97 g of hydroethanol extract (yield 1.39 %).

Estimation of Minimal Inhibitory Concentration (IC_{50}) Value

IC_{50} represents the amount of sample (µg extract/ml) necessary to scavenge free radicals by 50%. IC_{50} value was calculated from regression curve analysis plotting inhibition percentage against extract concentration.

Preliminary Phytochemical Analysis

Preliminary phytochemical tests were used to detect the presence of various organic functional groups, which are indicative of phytochemical types present in the plant. These tests indicated the different classes of constituents present in the extracts.

Determination of Total Phenolic Content

Phenolic compounds are plant secondary metabolites produced either from phenylalanine or from its precursor shikimic acid (Gilchrist *et al*., 1980). The antioxidant potential of phenolic compounds has been shown in a number of *in vitro* studies. The Folin-Ciocalteu (FC) method with some modification (Ikawa *et al*., 2003) was used to assess the phenolic content of the extracts. In this test, 100µl (0.1 mg/ml) of the extracts were mixed with 5 ml of the FC reagent (previously diluted with water at 1:10 v/v) and 4 ml of sodium carbonate (75 g/l). The tubes were vortexed for 15 sec and allowed to stand for 30 min at 40º C for colour

development. The absorbance was taken at 765 nm using spectrophotometer (MULTISKAN GO, Thermo Fischer Scientific). Total phenolic content was expressed at 0.1 mg/g gallic acid equivalent using the following equation based on calibration curve, y= 0.1216 x, where x = absorbance and y = gallic acid equivalent (mg/g).

Determination of Total Flavonoid Content

The aluminum chloride method (Chang *et al*., 2002) was used for the determination of the total flavonoid content of the extracts. In the test, 0.5 ml (0.2 mg/ml) of different extracts were added to the same amount of the 2% $AlCl_3$ ethanol solution and incubated for 1 hour at room temperature. Absorbance at 415 nm was recorded. A standard calibration plot was generated at 415 nm using known concentrations of quercetin. The concentrations of total flavonoid content was calculated using the following equation based on calibration curve, y=0.0255x, where x= absorbance and y = quercetin equivalent (mg/g).

High Performance Thin Layer Chromatography (HPTLC) Profile

The primary phytochemical analysis touted the presence of flavonoid and polyphenols in the EE, HE extract of *C. indicum*. Hence, all the above stated fractions were subjected to HPTLC study to detect and quantify the presence of the gallic acid and quercetin with the aid of CAMAG HPTLC System (Switzerland). Plant samples were dissolved in methanol to prepare the concentration of 10 mg/ml. Standards were prepared in the same solvent as 0.5 mg/ml. Then, 3 µl of each samples and standards were spotted on the 10×10 Silica Gel F_{60} using CAMAG LINOMAT 5 applicator. Mobile phase was applied as methanol: ethyl acetate: formic acid (2:6:0.2) and spotted plate was subjected to develop in the CAMAG ADC2 automated developing chamber. After drying the plate, it was scanned in CAMAG TLC Scanner employing long UV wavelength, 366 nm. All the solvents used in the experiment were of HPLC grade.

Analysis of Extracts by High Performance Liquid Chromatography-Diod Array Detection (HPLC-DAD)

The EE extract of *C. indicum* exhibited higher content of phytochemicals than HE extract of the plant. This result was also supported by HPTLC study. So, EE extract of *C. indicum* was analyzed in an HPLC system (Dionex, UHPLC 3000) using photodiode array detector (Dionex, UHPLC 3000). Separation was achieved using a reversed phase column, C18 (Acclaim® 120, 4.6×250mm, 4µm) at temperature of 25°C. DAD detection was employed at the wavelength range between 210 and 500 nm. The sample was dissolved in the corresponding solvent of the extract at 10 mg/ml. The volume of sample injected was 20 µl using an L-7200 autosampler. The mobile phase was a mixture of methanol: acetonitrile: water (60:20:20 v/v) (A) and 0.1% O-phosphoric acid: acetonitrile (400:600 v/v) (B) and the flow rate was 1 ml/min. The elution system was in isocratic mode. Chromatogram was analysed using Chromeleon software.

ANTIOXIDANT ACTIVITY ANALYSIS

Hydroxyl Radical Scavenging Activity

Generation of hydroxyl radicals is crucial for the irreversible damage in the tissue inflicted by oxidative stress (Orrenius *et al*., 2007).The hydroxyl radical scavenging ability was determined using Fenton reaction (Durre *et al*., 2012). This reaction mixture containing 200 µl of 2.8 mM deoxyribose (Sigma-Aldrich), 200 µl of 200 µM ferric chloride, 200 µl of 1.04 mM EDTA (Sigma-Aldrich), 200 µl of 1 mM ascorbic acid (HIMEDIA) and 200 µl of 1.0 mM H_2O_2 (Merck) were mixed with 1 ml of different concentrations of plant extracts and was allowed to incubate at 37°C for 1 hour. One ml of 1% thiobarbituric acid (TBA) (Sigma-Aldrich) and 1 ml of 2.8% trichloro acetic acid (TCA) (Sigma-Aldrich) were mixed with the reaction mixture and incubated for 15 min at 100°C in water bath. After cooling, the spectrophotometric readings were taken at 513 nm and percentage of inhibition was calculated according to the formula:

Percentage of inhibition = $[(Abs_{control} - Abs_{test}) / Abs_{control}] \times 100$.

Where $Abs_{control}$ is the absorbance of the control reaction and Abs_{test} is the absorbance in the presence of the sample.

DPPH Radical Scavenging Assay

In order to appraise the antioxidant potential through free radical scavenging of the test samples, the change in absorbance of DPPH radicals was done (Sanchez *et al*., 1998). In the experiment, 3 ml of reaction mixture comprised of 200 µl of 100 µM DPPH (Sigma-Aldrich) (in methanol) and 2.8 ml of samples at different concentrations. Free radical scavenging activity of the samples was expressed according equation:

Percentage of inhibition = $[(Abs_{control} - Abs_{test}) / Abs_{control}] \times 100$.

Where $Abs_{control}$ is the absorbance of the control reaction and Abs_{test} is the absorbance in the presence of the sample. All analyses were performed in triplicates.

Superoxide Radical Scavenging Activity (SOD)

Although superoxide anion is a weak oxidant, it ultimately yields potent oxidants such as hydroxyl radicals and singlet oxygen, both of which contribute to oxidative stress (Meyer *et al*., 1995). The superoxide anion scavenging activity can be measured using a standard protocol with some modifications (Robak *et al*.,

1988). The superoxide anion radicals are generated in 3.0 ml of phosphate buffer (100 mM, pH 7.4), 1 ml of nitroblue tetrazolium (NBT) (HIMEDIA) (156 mM), 1 ml NADH (HIMEDIA) (468 mM) solution and 3 ml of extracts with varying concentrations. The reaction was initiated by adding 1 ml phenazine methosulfate (PMS) (HIMEDIA) solution (60 µM) to the mixture, followed by incubation at 25° C for 5 min and then the absorbance was measured at 560 nm against a blank sample. The amount of inhibition was calculated using formula:

Percentage of inhibition = $[(Abs_{control} - Abs_{test}) / Abs_{control}] \times 100$.

Where $Abs_{control}$ is the absorbance of the control reaction and Abs_{test} is the absorbance in the presence of the sample. All analyses were performed in triplicates.

Reductive Ability Assay

Reducing power assay is based on the principle that substances with reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric-ferrous chromogenic complex (Jayprakash *et al*., 2001). In the assay, 1 ml of different concentrations of plant extracts was mixed with 2.5 ml of potassium ferricyanide (1%) and 2.5 ml of phosphate buffer (pH 6.6). The mixture was incubated at 50° C for 20 min. Then 2.5 ml of TCA (Sigma-Aldrich) (1%) was added and centrifuged at 3000 rpm for 10 min. From the above solution, 2.5 ml of the supernatant was taken. To 2.5 ml of water, 0.5ml of $FeCl_3$ (1%) was added and absorbance was taken at 700 nm using spectrophotometer. Ascorbic Acid was used as standard. The percentage of antioxidant activity was calculated as:

% antioxidant activity = $[(Abs_{control} - Abs_{test}) / Abs_{control}] \times 100$.

Where $Abs_{control}$ is the $Abs_{absorbance}$ of the control reaction and Abs_{test} is the absorbance in the presence of the sample. All analyses were performed in triplicates.

Statistical Analysis

Triplicate analyses were performed on all the samples. All numeric data were expressed as mean ± S.E.M. The graphs were prepared using Graph Pad Prism 6 (version 6.02). The effects of ethanolic and hydroethanolic extracts of *C. indicum*, both treatment and concentrations wise, on the activities exhibited were analysed by Two-way analysis of variance (ANOVA) followed by LSD using PASW 18.0 statistics (SPSS Inc.). $p<0.05$ was considered to be statistically significant.

RESULTS

Preliminary Phytochemical Analysis

The preliminary phytochemical analysis of plant ethanolic (EE) and hydroethanolic (HE) extracts of *C. indicum* divulged the presence of different phytoconstituents which is presented in Table1.

Determination of Total Phenolic Content

The antioxidant activities of the different extracts of *C. indicum* could be due to the presence of the phenolic compounds and flavonoids. The total phenolic content of the different extracts viz. EE and HE was found to be 49.934±2.43 mg/g and 39.723±1.84 mg/g respectively (Table 1).

Estimation of Total Flavonoid Content

Flavonoids are a large class of benzo-pyrone derivatives, omnipresent in plants and are responsible for the antioxidant property. Both the extracts of *C. indicum* reasonably showed appreciable flavonoid content. The EE extract of the plant touted the higher content of the flavonoid 10.235±3.63 mg/g with respect to the standard quercetin. The HE extract showed the flavonoid of 8.273±1.23 mg/g (Table 1).

HPTLC Profile

Under the chromatographic conditions described above, the R_f values and areas of the standard gallic acid and quercetin were 0.74, 0.84 and 3726.9 AU, 20420.2 AU respectively. The EE and HE extract of the plant of *C. indicum* showed the presence of quercetin, R_f values and areas were 0.88, 0.84 and 7639.8 AU, 3816.9 AU respectively (Table 2). Gallic acid was absent in the EE and HE extract of *C. indicum* (Figure 1).

HPLC-DAD Analysis

Reversed-phase column C-18 and mobile phase used in HPLC-DAD, was appropriate to the characterization of the EE extract. Figure 2 shows chromatogram at 273 nm of the extract, which contains flavonoids and phenolic acid derivatives in different proportions. Chromatographic analysis revealed the presence of flavonoid, quercetin in the EE extract of *C. indicum*. Quercetin showed the retention time of 3.36 min. (Fig. 2.)

Hydroxyl Radical Scavenging Activity

Ferric-ascorbate-EDTA-H_2O_2 (Fenton Reaction) yields the hydroxyl radicals which react with deoxyribose to produce thiobarbituric acid reactive substances (TBARS) and develops the pink chromogen in presence of thiobarbituric acid upon heating. The hydroxyl scavengers diminish the TBARS production and pink color formation by competing with deoxyribose for hydroxyl radicals. The hydroxyl radical scavenging activity of different extracts of *C. indicum* has been shown in the Figure 3. Both EE and HE exhibited hydroxyl scavenging activity in which EE extract exhibited the highest hydroxyl radical scavenging activity (IC_{50} 51.465 µg/ml) in comparison to standard antioxidant ascorbic acid (IC_{50} 24.573 µg/ml). The hydroethanolic extract of the *C. indicum* gave the lower

hydroxyl scavenging activity (IC_{50} 58.89 µg/ml) than EE.

DPPH Radical Scavenging Activity

DPPH radical scavenging activity of the different extracts of *C. indicum* was estimated using the standard procedure depicted earlier. In the present study, the DPPH radical reduction was observed in a concentration dependent manner. Higher DPPH scavenging activity was observed in the EE in respect to HE. The EE showed the IC_{50} of 49.52 µg/ml (Figure 4), whereas, HE extract showed an IC_{50} value of 82.17 µg/ml. However, ascorbic acid showed IC_{50} value of 4.59 µg/ml.

Superoxide Radical Scavenging Activity (SOD)

Superoxide is transformed to a more potent oxidative species, singlet oxygen and hydroxyl radicals (Lobo *et al*., 2010) in our body and renders adverse effects. The superoxide scavenging activity of *C. indicum* has been illustrated in Figure 5. The EE showed the higher superoxide scavenging activity (IC_{50} 64.06 µg/ml) in comparison to HE (IC_{50} 75.01 µg/ml).

Reductive Ability

Similar to the antioxidant, the reductive ability of the plant extracts increased with the increasing concentration. To investigate the reductive ability, Fe^{3+} to Fe^{2+} transformation was assessed in the presence of EE, HE of *C. indicum.* According to Figure 6, EE of *C. indicum* showed the higher reductive power of 0.49230 at 600 µg/ml concentration in respect to the HE extract which exhibited reductive ability of 0.31930 at 600 µg/ml concentration. Ascorbic Acid showed the reductive ability of 3.120 at 600 µg/ml concentration.

Table 1. Total Phenolic and Flavonoid content of the EE, HE extracts of *C. indicum*.

SL. No.	Phytochemical Tests	Qualitative Analysis		Quantitative Analysis	
		EE	HE	EE	HE
1	Steroids	+++[a]	++[b]		
2	Phenols	+++	++	49.934±2.43	39.723±1.84
3	Flavonoid	+++	++	10.2347±3.63	8.273±1.236
4	Tannin	++	++		
6	Glycoside	+[c]	-[d]		
7	Diterpenes	+	+++		
8	Triterpenes	+	+		

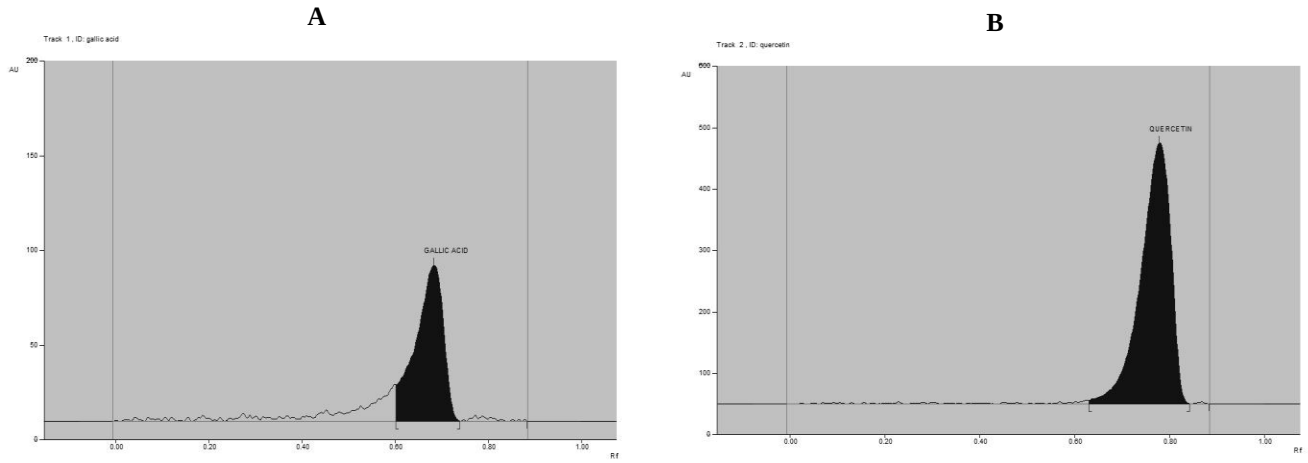
Note: a: (+++)- Highly present, b: (++)- Moderately present, c: (+)- Present, d: (-)-Absent.

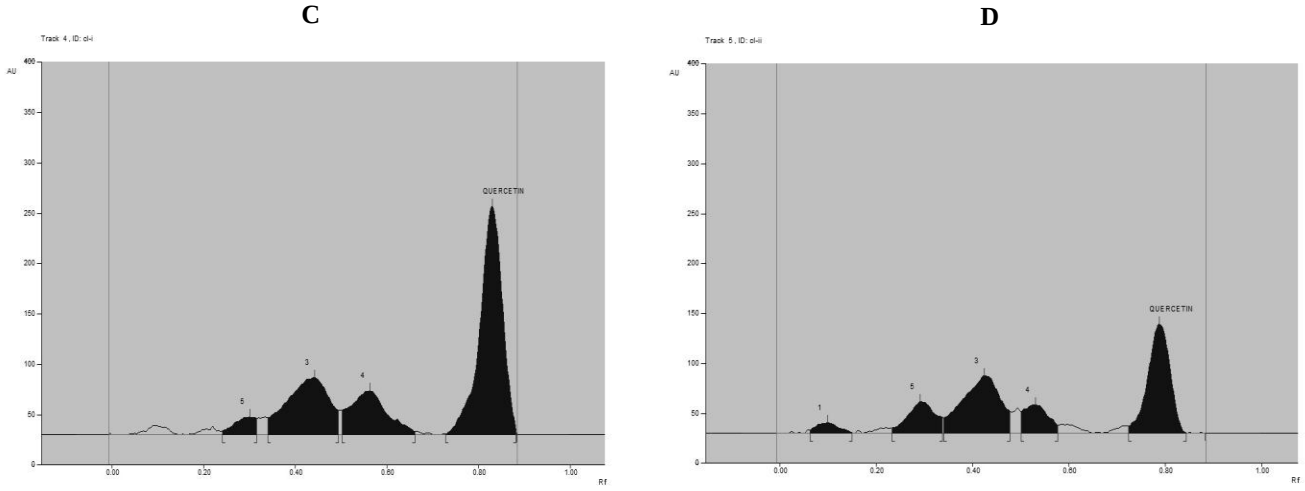
Table 2: R_f value and Area of the EE and HE extracts of *C. indicum* in HPTLC analysis.

Samples	Rf value		Area	
	Gallic acid	Quercetin	Gallic acid	Quercetin
Standard	0.74	0.84	3726.9 AU	20420.2 AU
EE extract	-	0.88	-	7639.8 AU
HE extract	-	0.84	-	3816.9 AU

Note: respective R_f values and areas of gallic acid, quercetin, EE and HE extracts.

Fig. 1. HPTLC chromatogram at 366 nm





a) Chromatogram of gallic acid (Rf 0.74).
b) Chromatogram of quercetin (Rf 0.84).
c) Chromatogram of EE extract of *C. indicum.*
d) Chromatogrm of the HE extract of *C. indicum*

Fig. 2. Chromatogram of the EE extract of *C. indicum* by HPLC-DAD method at 273 nm. It showed the presence of quercetin in EE extract (retention time 3.357 min)

Sample Name:	NST-GB-Cl 1	Injection Volume:	20.0
Vial Number:	154	Channel:	UV_VIS_1
Sample Type:	unknown	Wavelength:	273.0
Control Program:	12072012 GHILLA	Bandwidth:	4
Quantif. Method:	std Q	Dilution Factor:	1.0000
Recording Time:	24-09-2012 12:53	Sample Weight:	1.0000
Run Time (min):	8.00	Sample Amount:	1.0000

No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount ppm	Type
1	2.50	n.a.	696.197	384.236	81.13	n.a.	BM
2	3.36	Quercetin	26.838	3.938	0.83	0.001	Rd
3	4.58	n.a.	42.955	14.555	3.07	n.a.	Rd
4	5.82	n.a.	105.001	65.770	13.89	n.a.	MB
5	6.34	n.a.	9.503	2.184	0.46	n.a.	Rd
6	7.06	n.a.	10.448	2.922	0.62	n.a.	Rd
Total:			890.943	473.605	100.00	0.001	

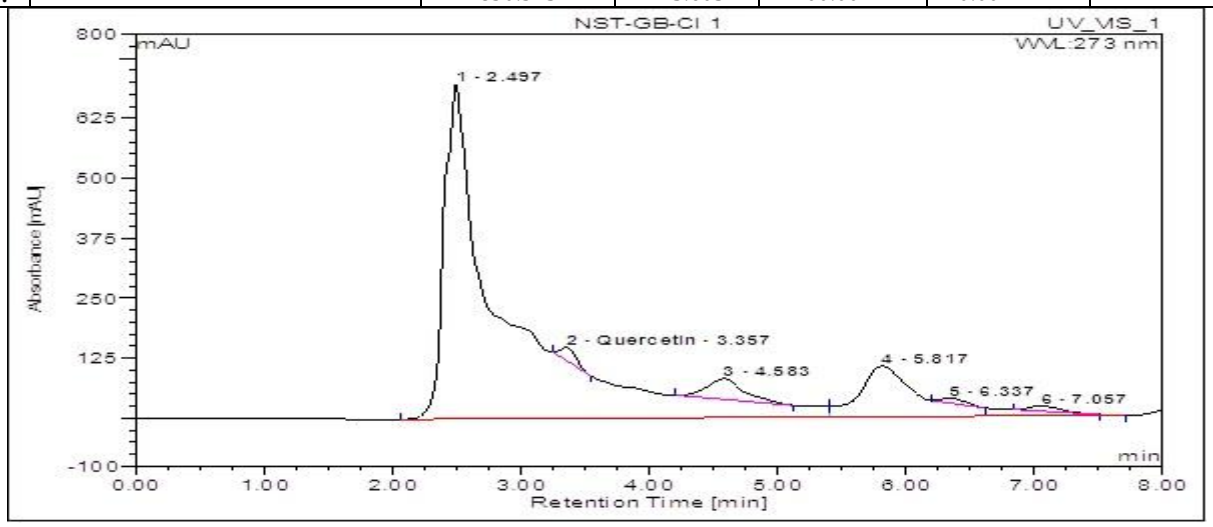


Fig. 3. Hydroxyl radical scavenging activity of ethanolic (EE), hydroethanolic (HE) extracts of *C. indicum.*

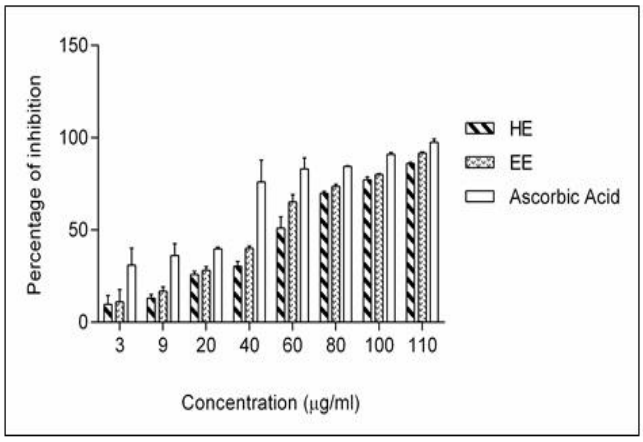


Fig. 4. DPPH scavenging activity of EE and HE extracts of *C. indicum.*

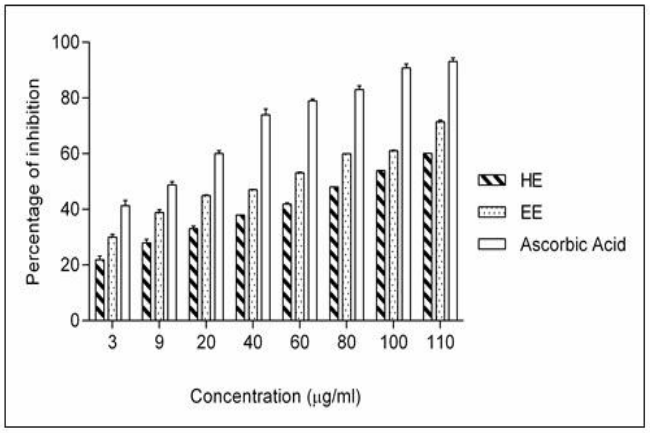


Fig. 5. Superoxide scavenging activity of EE and HE of *C. indicum.*

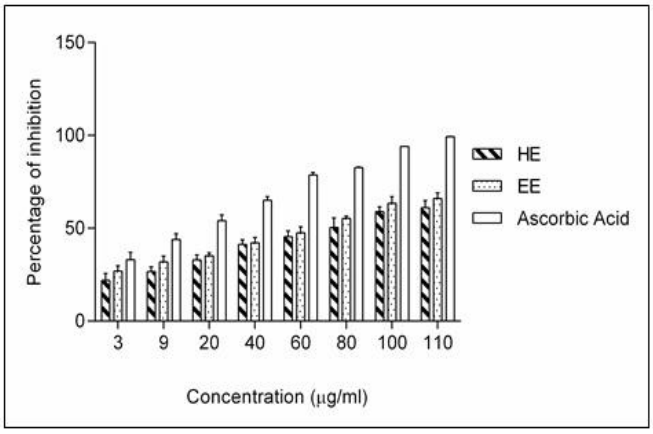
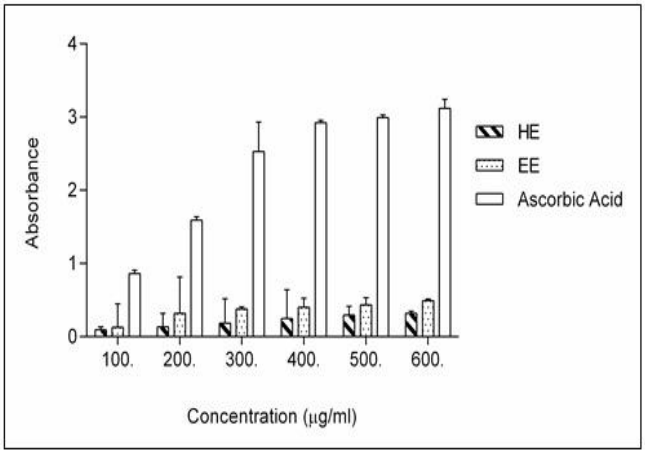


Fig. 6. Reductive ability of the EE and HE of *C. indicum.*



DISCUSSION

Polyphenols and flavonoids are the plant derived secondary metabolites, which significantly confer protection against development and progression of many chronic pathological diseases including cancer, diabetes, cardio-vascular problems and aging via their antioxidant activity (Pandey *et al*., 2007). The EE and HE extracts of *C. indicum* were assessed for the presence of their phytochemical constituents. The more active EE fraction showed the presence of steroids, phenols, favonoids, tannins, glycosides, di and tri-terpenes. The HE extracts was devoid of glycosides and exhibited lower amount of the phytochemical constituents in comparison to the EE extract of the plant. HPTLC analysis delineated the presence of quercetin in a concentration dependent manner declining from EE to HE extracts. In this regard, HPLC study was performed by taking the most active EE fraction. HPLC study supported the presence of quercetin in EE extract. It was reported that plant polyphenols (Zenebe *et al*., 2001) and flavonoids (Amic *et al*., 2003) play as antioxidants and render protection against diseases in our body. Moreover, a positive co-relation between total phenolic content and free radical scavenging activity has been reported (Oki *et al*., 2002)**.** The crude ethanolic extract of leaves of *C. indicum* attributed with antinociceptive activity due to presence of polyphenols, tannin and saponin (Raihan *et al*., 2012). Ethanol extract of *C. vicosum* (Prasanth *et al*., 2012) root and methanol extract of *C. infortunatam* (Das *et al*., 2010) have been reported to have anti-inflammatory activities due to presence of polyphenols and flavonoids. In this context, it can be said that, the polyphenols and flavonoids present in the EE and HE extracts of the *C. indicum* play crucial role not only as antioxidants but also diverse pharmacological properties as mentioned earlier. Our studies on such activities clearly showed positive results in this direction.

The hydroxyl radical is not only responsible for direct lipid peroxidation but also partakes in DNA damage which leads to mutagenesis, carcinogenesis and cytotoxicity (Li *et al*., 1998). The hydroxyl radical has a very ephemeral *in vivo* half-life of approximately 10^{-9} seconds and a high reactivity (Sies *et al*., 1993),

which makes it a very precarious compound to the organism (Reiter *et al*., 1995; Reiter *et al*., 1997). The EE extract of the plant body of *C. indicum*, is a better hydroxyl radical scavenger than the HE extract of the plant as EE extract showed greater content of both polyphenolics and flavonoid in phytochemical screening as well as HPTLC profile. The molecule, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) is characterized as a stable free radical because of its delocalized spare electrons which gives the intense violet colour responsible for giving maximum absorption spectra at 517 nm. Study has also revealed that, there is a positive correlation between the polyphenolic and flavonoid content of the plant and its DPPH activity. The EE extract rendered greater DPPH scavenging activity attributed by its higher polyphenol and flavonoid content than the HE extract of *C. indicum*. Superoxide anion is an initial free radical and a weak oxidant which ultimately produces stronger oxidative species such as singlet oxygen species and hydroxyl radicals (Stieff, 2003). Both EE and HE extract of *C. indicum* showed potent superoxide radical scavenging activity. The EE extract exhibited the higher activity than HE extract of the plant. Thus the phytochemical content of the plant extracts was reflected in the reductive ability of the EE and HE of *C. indicum*.

CONCLUSION

Thus the finding of our present study claims that the extracts of *C. indicum* wielding ethanol as solvent, attributed the highest antioxidant and free radical scavenging activity. The strong antioxidant activity could be due to its higher content of flavonoids like quercetin. It was obvious that, EE extract showed absence of gallic acid but phytochemical screening exhibited the higher content of phenolic compounds. Therefore, it can be concluded that, there are other types of phenolics rather than gallic acid present in the EE extract. The HE extract exhibited lesser degree of phytochemical content and antioxidant property than EE. In conclusion, ethanolic extract (EE) of *C. indicum* plant can be used as repertoire of natural antioxidants with consequent health benefits. However, further studies can elucidate the mechanism of action of the plant as an antioxidant and other unexplored medicinal properties.

ACKNOWLEDGEMENT AND FUNDING

We would like to thank the Department of Biotechnology, Ministry of Science and Technology, Government of India for their financial support and the Director of Research (Vety.), College of Veterinary Sciences, Khanapara for providing the necessary facilities.

CONFLICT OF INTEREST

Authors declare that the paper has no conflict of interest.

REFERENCES

Amic D, Amic DD, Beslo D, Trinajstic N. Structure-radical scavenging activity relationships of flavonoids. *Croat. Chem. Acta*, 76(1), 2003, 55-61.

Bunluepuech K, Tewtrakul S. Anti - HIV-1 integrase activity of Thai medicinal plants. Songklanakarin. *J. Sci. Technol*, 31(3), 2009, 289-292.

Butterfield AD. Commentary: Alzheimer's disease: a disorder of oxidative stress. *Alzheimer's Dis. Rev,* 1(1), 1996, 68-70.

Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimeric method. *J. Food Drug Analysis*, 10(3), 2002, 178-182.

Chen C, Pearson AM, Gray JI. Effects of synthetic antioxidants (BHA, BHT and PG) on the mutagenicity of IQ-like compounds. *Food Chem*, 43(3), 1992, 177-183.

Chiurchiù V, Maccarrone M. Chronic Inflammatory disorders and their redox control: from molecular mechanisms to therapeutic opportunities. *Antioxid. Redox Signal*, 15(9), 2011, 2605–2641.

Das S, Haldar PK, Pramanik G, Suresh RB. Evaluation of anti-inflammatory activity of *Clerodendron infortunatum* Linn. extract in rats. *Global J. Pharmacol*, 4(1), 2010, 48-50.

Devasagayam TPA, Boloor KK, Ramsarma T. Methods for estimating lipid peroxidation: Analysis of merits and demerits. *Indian J. Biochem. Biophys*, 40(2), 2003, 300-308.

Dizdaroglu M. Chemistry of free radical damage to DNA and nucleoproteins, in: DNA and Free Radicals, Halliwell, B., Aruoma, O. I. (Eds.), Ellis Horwood, London, 1993.

Droge W. Free radicals in the physiological control of cell function. *Physiol. Rev*, 82(1), 2002, 47–95.

Durre S, Muhammad AR, Afifa S, Madiha R, Faiza IC, Merva J, Sami U, Saif U. Antioxidant potential of the extracts of Putranjivaroxburghii, *Conyza bonariensis*, *Woodfordia fruiticosaand, Senecio chrysanthemoids*. *Afr. J. Biotech*, 11(18), 2012, 4288-4295.

Geldeman KA, Hultqvist M, Olsson LM, Bauer K, Pizzolla A, Olofsson P, Holmadhl R. Rheumatoid arthritis: the role of reactive oxygen species in disease development and therapeutic strategies. *Antioxid. Redox Signal*, 9(10), 2007, 1541-1567.

Gilchrist D, Kosuge T. Aromatic amino acid biosynthesis and its regulation, *in:* Miflin, B. N. (Eds), The biochemistry of plants, Academic Press, New York, 1980.

Ikawa M, Schaper TD, Dollard CA, Sasner JJ. Utilization of Folin–Ciocalteu phenol reagent for the detection of certain nitrogen compounds. *J. Agric. Food Chem,* 51(7), 2003, 1811–1815.

Jayprakash GK, Singh RP, Sakariah KK. Antioxidant activity of grape seed extracts on peroxidation models *in-vitro*. *J. Agric. Food Chem,* 73(1), 2001, 285-290.

Kunwar A, Priyadarsini KI. Free radicals, oxidative stress and importance of antioxidants in human health. *J. Med. Allied. Sci,* 1(2), 2011, 53–60.

Li W, Sun GY. Polyphenolic antioxidants on cytokine-induced iNOS and $sPLA_2$ in an immortalized astrocyte cell line (DITNC), in: Packer, L., Ong, A.S.H. (Eds.), Biological oxidants and antioxidants: molecular mechanisms and health effects, AOCS Press, Champaign, 1998.

Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacog. Rev,* 4(8), 2010, 118–126.

Manandhar NP, Manandhar S. The useful plants of Nepal, in: Manandhar NP, Manandhar S. (Eds.), Plants and people of Nepal, Timber Press, London, UK, 2002.

Meyer AS, Isaksen A. Application of enzymes as food antioxidants. *Trends Food Sci. Tech,* 6(1), 1995, 300-304.

Naskar S, Mazumder UK, Pramanik G, Bala A, Haldar PK, Islam A, Gupta M. Comparative *in vitro* antioxidant activity of different parts of *Cocos nucifera* (Linn.) on reactive oxygen and nitrogen species. *Int. J. Pharm. Sci,* 3(3), 2011, 104-107.

Oki T, Masuda M, Furuta S, Nishibia Y, Terahara N, Suda I. Involvement of anthocyanins and other phenolic compounds in radical-scavenging activity of purple-fleshed sweet potato cultivars. *Food Chem. Toxicol,* 67(5), 2002, 1752-1756.

Orrenius S, Gogvadze V, Zhivotovsky B. Mitochondrial Oxidative Stress: Implications for Cell Death. *Ann. Rev. Pharmacol. Toxicol,* 47(3), 2007, 143-183.

Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. *Physiol. Rev,* 87(1), 2007, 315-424.

Pal A, Zobaer AM, Akter N, Islam MS, Bachar SC. Evaluation of antinociceptive, antidiarrheal and antimicrobial activities of leaf extracts of *Clerodendrum indicum*. *Pharmacog. J*, 30(2), 2012 41-46.

Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidant in human health and disease. *Oxid. Med. Cell Longev,* 2(5), 2009, 270–278.

Prasanth KG, Anandbabu A, Johns T, Dineshkumar B, Krishnakumar K, Geetha G, Venkatanarayana R. Ethanol extract of *Clerodendrum viscosum* vent Roots: Investigation of analgesic and anti inflammatory effects in male adult Swiss albino mice. *Int. J. Nat. Prod. Res,* 1(4), 2012, 67-71.

Rahman MZA, Azam ATMZ, Gafur MA. Favonoids from *Clerodendrum indicum* Linn. *Pak. J. Biol. Sci,* 3(3), 2000, 1769-1771.

Raihan SZ, Biswas P, Monir MdM, Biswas SK, Chowdhury A, Sahidur Rahaman AKM. Phytochemical investigation and *in vitro* antinociceptive activity of *Clerodendrum indicum* leaves. *Pak. J. Biol. Sci,* 15(3), 2012, 152-155.

Reiter RJ, Carneiro RC, Oh CS. Melatonin in relation to cellular antioxidative defense mechanisms. *Horm. Metab. Res,* 29(8), 1997, 363–372.

Reiter RJ, Melchiorri D, Sewerynek E, Poeggeler B, Barlow-Walden L, Chuang J, Ortiz GG, Acuña-Castroviejo D. A review of the evidence supporting melatonin's role as an antioxidant. *J. Pineal. Res,* 18(1), 1995, 1-11.

Robak J, Gryglewski RJ. Flavonoids are scavengers of superoxide anions. *Biochem. Pharmacol,* 37(5), 1988, 837-841.

Sanchez-Moreno C, Larrauri JA, Saura-Calixto FA. Procedure to measure the anti-radical efficiency of polyphenols. *J. Agric. Food Chem,* 76(43), 1998, 270-276.

Seifried HE, Anderson DE, Fisher EI, Milner JA. A review of the interaction among dietary antioxidants and reactive oxygen species. *J. Nutr. Biochem,* 18(9), 2007, 567–579.

Shrivastava N, Patel P. Clerodendrum and Heathcare: An Overview. *Medicinal Aromatic Plant Sci. Biotech,* 1(1), 2007, 142-150.

Sies, Helmut. Strategies of antioxidant defense. *Eur. J. Biochem,* 215(2), 1993, 213–219.

Slemmer JE, Shacka JJ, Sweeney MI, Weber JT. Antioxidants and free radical scavengers for the treatment of stroke, traumatic brain injury and aging. *Curr. Med. Chem,* 15(4), 2008, 404-414.

Stief TW. The physiology and pharmacology of singlet oxygen. *Med. Hypotheses,* 60(4), 2003 567-572.

Winyard PG, Zhang Z, Blake DR. Reactive oxygen species and their adverse effect, in: Thomas, CE, Kalyanaraman B. (Eds.), Oxygen Radicals and Disease Process, Hardwood Academic Publishers, The Netherlands, 1998.

Zenebe W, Pechánová O, Bernátová I. Protective effects on red wine polyphenolic compounds on the cardiovascular system. *Exp. Clin. Cardiol,* 6(3), 2001, 153–158.