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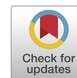
Original article

Insights of antidiabetic, anti-inflammatory and hepatoprotective properties of antimicrobial secondary metabolites of corm extract from *Caladium x hortulanum*



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ABSTRACT

Medicinal plants have therapeutic potential and are used worldwide to treat various diseases. In this study, the corm of *Caladium x hortulanum* was extracted with various solvents and implied the availability of phytochemicals such as flavonoids, alkaloids, tannins, steroids, phenols, glycosides, saponins and terpenoids. The solvent extracts of the corm showed antibacterial and antifungal activity with the growth inhibition zone ranged 0–24 mm. The isolation of phytochemicals was carried out using gel column chromatography, Thin Layer Chromatography followed by High Performance Liquid Chromatography. Gas Chromatography and Mass Spectrophotometry analysis was used to determine the phytochemicals. The corm extract showed potent antidiabetic activity on Hep G2 cell lines and CCl_4 induced toxicity was elucidated. This possessed antiinflammatory property on murine monocyclic macrophage cell line RAW 264.7 showed 45.85 ± 1.8% inhibition of cyclooxygenase activity. The corm extract showed hepatoprotective activity. The CCl_4 incorporated Hep G2 cells showed 19.629 ± 1.5% viability, whereas viability increased as 78.82 ± 1.9% at 100 μg/ml of extract.

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1. Introduction

The medicinal uses of plants have continued to be a good source of natural products for treating various diseases. It is estimated that more than 150,000 plant species were studied and many of them contain valuable therapeutic agents and the applications of novel compounds from plant for pharmaceutical purposes has been gradually increasing in recent years. In developing countries, a major population still uses traditional folk medicine obtained from medicinal plants (Farnsworth, 1994; Srivastava et al., 1996; Castello et al., 2002). India is represented by rich tradition, good biodiversity and it offers good opportunity for drug research (Jachak and Saklani, 2007). Numerous traditional natural products have been increased in recent years and much work has been carried out on selected medicinal plants for antimicrobial activity against various pathogenic strains of Gram positive and Gram negative bacteria (Singh et al., 2002). Many works have been done aiming to understand the different phytochemical and antimicrobial constituents of plants and using them for the treatment of various bacterial diseases as possible alternatives to synthetic drugs to which many pathogenic microorganisms have become resistant against broad spectrum antibiotics. The use of natural products over conventional treatment of various diseases has been increased in the last few decades Ansari et al., 2006).

The drug resistant human pathogenic bacteria have been reported widely throughout the world (Piddock and Wise, 1989; Singh et al., 1992; Mulligen et al., 1993; Davis, 1994; Robin et al., 1998). Moreover, the situation is alarming, in developed and many developing countries mainly due to excess use of antibiotics. Hence, the search of novel antimicrobial agents from plant sources continues (Basualdo et al., 2007). According to WHO (2001) medicinal plants would be the suitable source to obtain novel drugs. These medicinal plants are the rich resource of food supplements, folk's medicine, chemical entities, pharmaceutical intermediates and modern medicine (Ncube et al., 2008). The important

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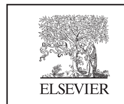

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bio-active constituents of plants are tannins, alkaloids, saponins, flavonoids, glycosides, terpenoids, phenolic compounds and steroids (Edeoga et al., 2005). These natural metabolites of most of tropical medicinal plants may possess a potent source of antimicrobial agents with novel properties (Ahmad and Aqil, 2007). The antimicrobial properties of these medicinal plants have been reported widely in recent years (Grosvenor et al., 1995; Nimri et al., 1999). These medicinal plants showed potent activity against various human pathogenic bacteria (Molla et al., 2011; Panghal et al., 2011; Mwitari et al., 2013; Bishnu et al., 2015).

The parts of medicinal plants such as, stem, root, fruit, flower, seed etc. are widely used to obtain various pharmacologically active substances. These active substances are mainly isolated for direct application as drugs. Diabetes mellitus is a disorder of metabolism that is characterized by hyperglycemia (Chandra et al., 2004). In recent days, herbal plants are used worldwide even without documentation of their curative effect against various diseases and there is only little information known about the pharmacological potential of many plants used in the traditional medicine system (Gupta et al., 2008). And, various investigations stated that oral anti-hyperglycemic agents derived from medicinal plants can be used in traditional medicine and many of the medicinal plants were found with potential antidiabetic properties (Kesari et al., 2007). In an another study, continuous oral administration of the *Cestrum nocturnum* leaves extract for 15 days caused a significant reduction in blood glucose levels in diabetic rats and considerable body weight was increased (Kamboj et al., 2013). It was also reported that diabetic rats treated with methanolic extracts of *Dolichos lablab* reduced blood triglycerides, SGOT, SGPT, total cholesterol and blood glucose levels in streptozotocin induced diabetic rat (Kante and Reddy, 2013). Similarly, acetone extract of whole fruit of *M. charantia* lowered the blood glucose from 13.3% to 50.0% in alloxan diabetic rats (Singh and Gupta, 2007). Diabetes mellitus is one of the metabolic disorders with macro-and macrovascular complications that results insignificant mortality. This disease is considered as one of the leading causes of death in the world (Vats et al., 2004). In modern medicine the treatment is not satisfactory so far to cure diabetes mellitus (Sumana and Suryawashi, 2001). The applications of oral hypoglycaemic agents and insulin have some side effects, hence there is increasing demand to search natural products with potential antidiabetic activity (Holman and Turner, 1991; Kameswara Rao et al., 2001; Kameswrarao et al., 1997). There are number of traditional medicinal plants have been reported to have hypoglycaemic properties such as *Azadirachta indica* (Neem), *Allium sativum* (Garlic), *Trigonella foenum* (Fenugreek), *Ocimum santum* (Tulsi), *Vinca rosea* (Nayantara) and *Momordica charantia* (Bitter ground). However, these plants are less effective in lowering blood glucose levels in severe diabetic patients. Fresh leaf extract of *Vinca rosea* has potent antidiabetic activity and reduce blood glucose in alloxan diabetic and normal rabbits (Nammi et al., 2003). Twigs and leaves of *Catharanthusroseus* have been registered to have potent hypoglycaemic activity (Singh et al., 2001).

Medicinal plants secrete secondary metabolites around their rhizosphere to repel or inhibit the activity of soil-born plant pathogens. The secondary metabolites such as, terpenoids and phenolics are two important classes of root-secreted metabolites having antimicrobial properties (Baetz and Martinoia, 2014). The phenolic compounds include, flavonoids and phenylpropanoids were the root-secreted secondary metabolites (Harborne and Williams, 2000). Phenylpropanoids were isolated from the root of various plants with potent antimicrobial activities. Also, these were produced by the root system of barley and inhibited the spore germination of *Fusarium graminearum* (Lanoue et al., 2010). Recently, Cao and Deng (2017) reported two unigenes that are involved in the synthesis of phenylpropanoid and four unigenes responsible for flavonoid synthesis. Flavonoids and terpenoids are the important groups of phenolic compounds in root exudates (Baetz and Martinoia, 2014; Vaughan et al., 2013). Some terpenoids are volatile and emitted from root system that could act as potential natural barrier against various soil pathogens (Baetz and Martinoia, 2014). In *Caladium*, four unigenes were recently identified from the root transcriptome which are take part in the synthesis of terpenoid (Cao and Deng, 2017). In *Arabidopsis*, a volatile compound, monoterpene 1,8-cineole was reported with potential antibacterial activity (Steeghs et al., 2004). Kapulnik et al. (2011) reported that the root system of some of the medicinal plants release strigolactones showed potential activity against *Fusarium solani*, *Fusarium oxysporum* and *Macrophomina phaseolina*. The present knowledge on phytochemical components, antimicrobial properties against various human pathogens, antidiabetic, antiinflammatory and hepatoprotective activity on *Caladium x hortulanum* is limited. Hence, the present investigation was performed to find the phytochemical constituents, antimicrobial activity, antidiabetic, antiinflammatory and hepatoprotective activities of the corm extract.

2. Materials and methods

2.1. Plant material and extraction

The plant, *Caladium x hortulanum* was collected along with corm from Kanyakumari District, Tamil Nadu, India. The collected sample was rinsed several times with tap water and dried in dark at room temperature (30 ± 2 °C) for 10 days. The corm was blend mechanically and finely powdered. This powdered material was used for the extraction of secondary metabolities using various solvents such as acetone, chloroform, dimethyl sulfoxide and ethanol. Individually 10 g of dried sample was used for secondary metabolite extraction with 500 ml of solvent for 3 h. The solvent portion was evaporated, and the sample was further analyzed.

2.2. Screening of phytochemicals from the corm

The protein content, carbohydrate content and phytochemical components such as alkaloids, tannin, vitamin C, flavonoids, phenols, sterioids, glycosides, terpenoids, saponins were screened (Raaman, 2006).

2.3. Bacterial strains and inoculum preparation

The antibacterial activity of corm extract was carried out using four bacterial and three fungal pathogens. The selected bacterial pathogens were *Klebsiella pneumonia, Escherichia coli, Staphylococcus aureus* and *Bacillus subtilis*. The fungal species such as, *Aspergillus fumigates, A. niger* and *Penicillium* sp. were used for antifungal activity screening. All bacterial pathogens were cultured in nutrient broth medium (Himedia, Mumbai, India). Fungal isolates were cultured in Potato Dextrose Broth and incubated at 37 °C for 48 h. The growth of the isolates was monitored at 600 nm using a UV–Visible spectrophotometer and the culture was appropriately diluted to attain 10^7 CFU/ml.

2.4. Antibacterial and antifungal activity of plants extract

In this study, well diffusion method was used to study the antimicrobial activity of the corm extract. Mueller-Hinton agar plates (g/l) (beef infusion – 300, casein acid hydrolysate – 17.5, starch – 1.5 and agar – 17.5) were prepared and used for antimicrobial screening. 20 μl of corm extract from different solvents were used in each well with gentamycin (30 μg) as the positive control. All plates were incubated at 2–8 °C for 5 h and further incubated at

37 °C for 24 and 48 h, respectively. The zones of inhibition were measured (in mm) and antibacterial antifungal activity was registered.

2.5. Isolation of secondary metabolite using column chromatography

Silica gel was used as stationary phase for column chromatography. The highly active fraction of the corm extract was further fractionated using silica gel chromatography employing an elution step (hexane 100%, hexane 75%: ethyl acetate 25%, hexane 50%: ethyl acetate 50% hexane 25%: ethyl acetate 75%). The fractions were (10 ml) collected and the active fraction was selected, dried and frozen at −80 °C.

2.6. Thin layer chromatography

The compound purified from silica gel column with the concentration of 1 mg/ml was spotted on a Thin Layer Chromatography plate (Merck, Bangalore, India) and dried. Chloroform and methanol (19:1) mixture was used as the mobile phase and the compounds were separated. The sample spots were visualized and the bands were scraped off individually from the TLC plates, transferred into separate vials and 1 ml acetonitrile was added to the sample.

2.7. Reverse phase HPLC (RP – HPLC) purification of secondary metabolites

Isolation of the molecule was performed using a RP – HPLC system (Cyberlab, USA). C-18 column was used and acetonitrile: water (65:35) was used as mobile phase. The solvents and samples applied in this system were sonicated for 30 min prior to HPLC. 10 μL of the TLC purified sample was applied and eluted at the rate of 1 ml/min.

2.8. Gas chromatography-Mass spectrometry (GC–MS) analysis

The GC–MS analysis of corm phytochemical was evaluated using silica column. About 1 μl of extract was injected and mass spectral scan range was set at 50–650 (*m/z*) and the ionization voltage was 70 eV. Helium was used as the carrier gas and the column (50 m × 0.25 mm) was fitted closely to a Perkin Elmer gas chromatography. This system was equipped with a detector. The analyzed conditions were, column temperature 235 °C for 3 min, injection temperature 240 °C and the split ratio was 5:4. The spectrum of the phytochemicals was compared with the database of spectrum of known components stored in the GC–MS NIST (2008) library.

2.9. Determination of invitro glucose uptake assay of C. x hortulanum extracts on culture Hep G2 cell lines

Hep G2 hepatic cells were purchased from NCCS Pune was maintained in Dulbecco's modified eagles media (HIMEDIA) supplemented with 10% FBS (Invitrogen) and grown to confluence at 37 °C in 5% CO_2 in a humidified atmosphere in a CO_2 incubator (NBS, EPPENDORF, GERMANY). The cells were trypsinized (500 μl of 0.025% Trypsin in PBS/0.5 mM EDTA solution (Himedia)) for 2 min and passed to T flasks in complete aseptic conditions. The cells were then subcultured in a 24 well plate. After attaining 80% confluence cells were kept in DMEM without glucose for 24 h. The purified compound from corm extract was added to grown cells at a final concentration of 6.25 μg, 12.5 μg, 25 μg, 50 μg and 100 μg from a stock (1 mg/ml) and incubated for 24 h in DMEM containing 300 mM glucose. An untreated control with high glucose was also maintained. After incubation, cells were isolated by centrifugation (6000 rpm, 10 min). Supernatant was discarded and 200 μl of cell lysis buffer (100 mM Tris HCl, 250 mM EDTA, 2 M NaCl, 0.5% Triton) was added. The incubation was carried out for 30 min at 4 °C and the glucose uptake was estimated using DNSA method. About 100 μl of sample was mixed with 100 μl of DNSA and placed on a boiling water bath for 10 min. After cooling, it was diluted with double distilled water and the OD was read at 600 nm using a UV visible spectrophotometer. All experiments were carried out in triplicates and mean value was used to find the glucose uptake (%) (Yap et al., 2007).

2.10. Determination of invitro antiinflamatory effect of root extracts on cultured RAW cell lines

The murine monocyclic macrophage cell line RAW 264.7 was maintained in Rose well Park Memorial Institute (RPMI) media (HIMEDIA, Mumbai, India) supplemented with 10% heat inactivated FBS, antibiotics (penicillin and streptomycin, 100 U/ml) and 1.5% sodium bicarbonate. The culture media was filter sterilized using 0.2 μm cellulose acetate filter paper. The cells were then grown till 60% confluence followed by activation with 1 μl lipopolysaccharide (LPS, 1 μg/ml). LPS stimulated cells were exposed with different concentrations of samples (6.25 μg/ml, 12.5 μg/ml, 25 μg/ml, 50 μg/ml and 100 μg/ml) from a stock (1 mg/ml) dissolved in 1% DMSO and incubated for 24 h. The anti inflammatory effects of samples were determined by assessing the inhibition of cyclooxygenase (COX) spectrophotometrically. 200 μl of cell lysis buffer (1 MTris HCl, 250 mM EDTA, 2 M NaCl, 0.5% Triton) was added to the pellet after centrifuging at 6000 rpm for 10 min. The incubation was carried out for 30 min at 4 °C and enzyme assay was performed with supernatant.

2.10.1. Cycloxygenase (COX) inhibition assay

The assay mixture contained assay buffer (Tris-HCl, 100 mM, pH 8.0), 5 mM GSH, 5 μM haemoglobin and 200 μM arachidonic acid. The reaction was started by the addition of arachidonic acid and stopped after 20 min of incubation at 37 °C by the addition of 0.2 ml of 10% Trichloro acetic acid (TCA). It was then mixed with 0.2 ml of 1% Thiobarbituric acid and the contents were heated in a boiling water bath for 20 min. It was then cooled and centrifuged at 1000*g* for 3 min. The supernatant was measured at 632 nm for COX activity (%) (Yap et al., 2007).

2.11. Determination of invitro hepatoprotective effect of corm extract on Hep G2 cell lines

Hepatoprotective activity screening was mainly based on the protection of human liver cells derived Hep G2 cells against cell damage induced by CCl_4 (Thabrew et al., 1997). Hep G2 hepatic cell lines was purchased from NCCS Pune were maintained in Dulbecco's modified eagles media (HIMEDIA, Mumbai, India) supplemented with 10% FBS (Invitrogen) and grown to confluence at 37 °C in 5% CO_2 in a humidified atmosphere in a CO_2 incubator (NBS, EPPENDORF, GERMANY). The cells were trypsinized (500 μl of 0.025% trypsin in PBS/0.5 mM EDTA solution (HIMEDIA, Mumbai, India) for 2 min and passed to T flasks in complete aseptic conditions. Extracts were added to grown cells at a final concentration of 6.25 μg/ml, 12.5 μg/ml, 25 μg/ml, 50 μg/ml and 100 μg/m land incubated for 24 h and the hepatoprotective effect of extract on CCl_4 induced damage was evaluated. CCl_4 (0.1%) was added to induce toxicity and the percentage (%) difference in viability was determined by using MTT assay after 24 h of incubation (Hu et al., 1999).

2.11.1. MTT cytotoxicity assay

The MTT cytotoxicity assay was carried out as described previously (Gacche et al., 2011). The liver cells were inoculated in 96 wells micro titer plates. After overnight incubation, cells were treated with corm extract for 1 h. The cells were washed with 1X phosphate buffered saline (PBS) and 30 μl of MTT solution was added to the culture (5 mg/ml) It was then incubated at 37 °C for 3 h and MTT was removed by washing with 1X PBS and 200 μl of DMSO. Incubation was carried out at room temperature for 30 min until the cells were lysed and colour was developed. The solution was then transferred to micro centrifuge tubes and centrifuged at 12,000 rpm for 2 min. Optical density was read at 540 nm using DMSO as blank in a microplate reader and viability (%) was calculated (ELISASCAN, ERBA) (Gacche et al., 2011).

3. Results and discussion

3.1. Phytochemicals of C. x hortulanum corm and its antimicrobial properties

In this study, phytochemical screening of the corm extract of *C. x hortulanum* was done to assess the availability of bioactive secondary metabolities. The presence of phytochemicals such as, flavonoids, alkaloids, tannins, steroids, phenols, glycosides, saponins and terpenoids were detected. Among these, phenols, alkaloids, flavonoids, tannins and saponins are important bioactive compounds and these are responsible for medicinal properties of this plant. The corm sample also was found to be rich of carbohydrates. Flavonoids are well known for health promoting properties such as anti-allergic, antioxidant, antimicrobial, anti-inflammatory and anticancer properties (Aiyelaagbe and Osamudiamen, 2009). Phenolic compounds showed potent antioxidant properties (Aliyu et al., 2009). Terpenoids such as sesquiterpenes, diterpenes and triterpenes have been referred to as insecticidal, antibiotics, antiseptic and anthelmintic in pharmaceutical industry (Parveen et al., 2010). Alkaloids have been widely reported to possess analgesic, bactericidal, antimalarial and analgesic activities (Okwu and Okwu, 2004). Recently, Ekanem et al. (2013) analyzed the phytochemical content of different parts of *Caladium* sp. and found significant levels of alkaloids, flavonoids and saponins in the leaves. In medicinal plants these secondary metabolites greatly contribute towards the biological activities such as antidiabetic, hypoglycemic, antimicrobial, antiinflammatory, antioxidant, antimalarial, anticarcinogenic and antileprosy activities (Negi et al., 2011). The plant of concern for screening was found to have more flavonoids. These flavonoids have immense bioactive potential. Flavonoids from the medicinal plants have anticancer property and also prevent oxidative cell damage (Rio et al., 1997). Flavonoids have antioxidant properties include protection against inflammation, allergies, platelet aggregation and free radicals (Barakat et al., 1993). The antimicrobial and antifungal activity of the *C. x hortulanum* corm extracts were assayed against selected bacteria and fungi. The present study revealed that all the solvent extracts tested have varying degree of antimicrobial and antifungal properties against the tested bacterial and fungal strains (point repeated). Based on the solvents tested, the antimicrobial properties varied. The corm extract exhibited potent activity against tested bacteria (*S. aureus*, *K. pneumoniae*, *E. coli* and *B. cereus*) and fungi (*Penicillium* sp., *A. niger* and *A. fumigates*) (Table 1). Terpenoids have highly useful in the therapy and prevention of various diseases, including cancer. These compounds are also possessed antifungal, antimicrobial, antiparasitic, anti-allergenic, antiviral, immunomodulatory and antihyperglycemic properties (Rabi and Bishayee, 2009). Terpenoids also have insecticidal properties and can be used as protective substances in storing agriculture products (Sultana and Ata, 2008). Saponins have antifungal properties and this ability towards microbes make them good candidates for treating yeast and fungal infections.

Table 1
Antibacterial and antifungal activity of corm extract from *C. x hortulanum* (– no activity).

Pathogenic strains	Ethanol	Chloroform	Acetone	Dimethyl sulfoxide
S. aureus	13	14	–	11
B. cereus	14	13	21	10
K. pneumoniae	13	12	12	12
E. coli	13	9	15	–
A. fumigatus	12	11	11	–
A. niger	11	12	18	12
Penicillium sp.	12	11	–	13

3.2. Purification of secondary metabolites for therapeutic applications in invitro

In this study, the corm extracted with acetone was further purified using silica gel column chromatography. Hexane (50%): ethyl acetate (50%) fraction showed potent antibacterial activity against tested bacterial and fungal pathogens. Among the bacteria, *B. cereus* showed more inhibitory effect (24 mm) and was very effective against *A. niger* (19 mm). Then this hexane – ethyl acetate fraction was subjected to TLC separation and bands were identified. The bands with similar Rf value was combined and concentrated. The concentrated sample was subjected to analyze its purity using a HPLC and a major peak was observed at 1.554 min (Fig. 1).

The GC–MS analysis of the extract of the corm of *C. x hortulanum* showed the presence of many phytochemicals which greatly contribute to the biological activity of this medicinal plant. In this study, GC–MS analysis showed the presence of 9 compounds from the acetone extract (Fig. 2). The important components determined in GC–MS analysis were, n-Hexadecanoic acid, phytol, octadecatrienoic acid and fatty acid methyl esters. It was reported that the presence of fatty acid methylesters (FAME) for the antifungal activities in medicinal plant extracts (Agoramoorthy et al., 2007). Chandrasekharan et al. (2008) elucidated the antimicrobial activity of medicinal plants from the family, Chenopodiaceae and reported that the methyl esters of *Salicornia brachiata* showed good antifungal potential. Recently, Lima et al. (2011) have reported antimicrobial properties of seeds of *Annona cornifolia* because of the availability of fatty acid methyl esters in high quantities. In this study, 1-ascorbic acid 2,6-dihexade canoate was also determined in the acetone extract of the corm. Naturally available ascorbic acid has anti-scorbutic activity and helps in absorption from the intestine. It is very essential for metabolism especially to bones, teeth and tissue (Akinmoladun et al., 2007). Steroids from the plants have potential antibacterial properties and tannins bind specifically on proline rich proteins and interrupt protein synthesis in microbes thus inhibit the growth of the organism (Epand, 2007).

3.3. Antidiabetic properties of secondary metabolities from the corm of C. x hortulanum

In this study, the antidiabetic activity of the corm extract of *C. x hortulanum* was tested in *invitro* condition using Hep G2 cell lines. Diabetes mellitus is a condition of hyper-production of glucose in individuals and less utilization by the tissues (Chattopadhyay, 1993). The cytotoxic effects of corm extract were tested on this cell lines. The corm extract was added to Hep G2 cells at various concentrations (6.25 μg/ml, 12.5 μg/ml, 25 μg/ml, 50 μg/ml and 100 μg/ml) and incubated for 24 h and the hepatoprotective effect of extract on CCl_4 induced toxicity was carried out. At 6.25% μg/ml,

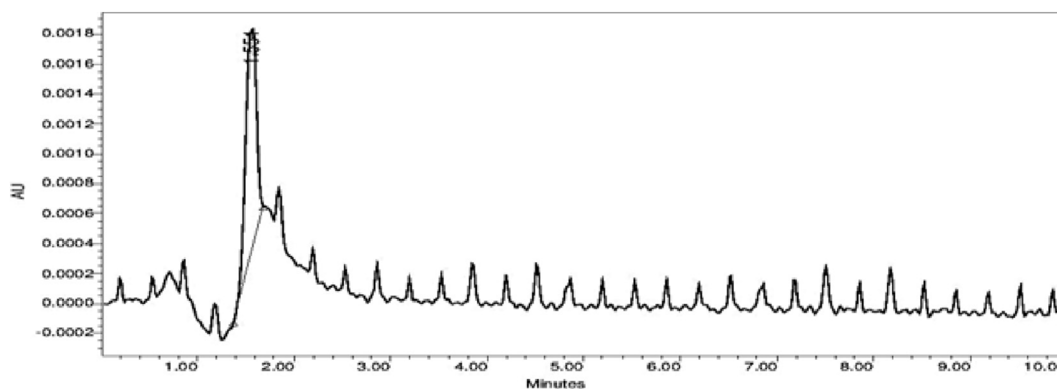

Fig. 1. Elution profile of secondary metabolities from the corm of *C. x hortulanum* in High Performance Liquid Chromatography (HPLC). A major peak was detected at 1.554 min and the elution was carried out up to 10 min.

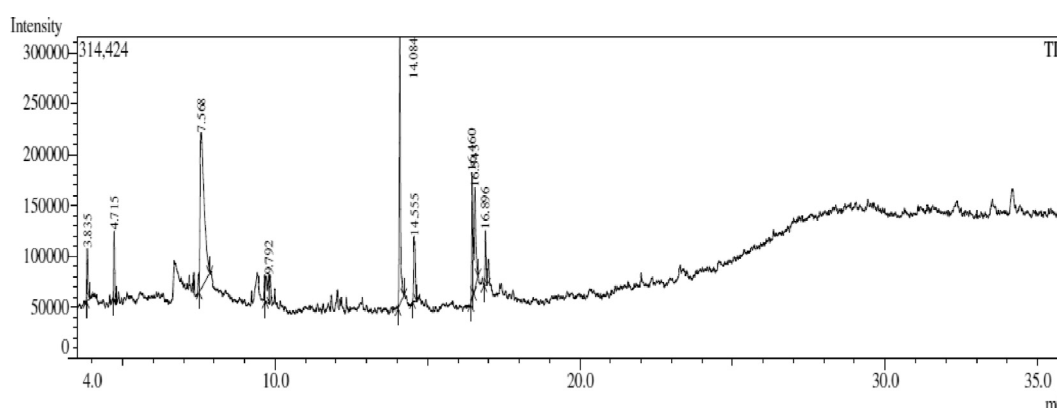

Fig. 2. Gas Chromatography – Mass Spectrophotometry profile of secondary metabolites from the corm of *C. x hortulanum.*

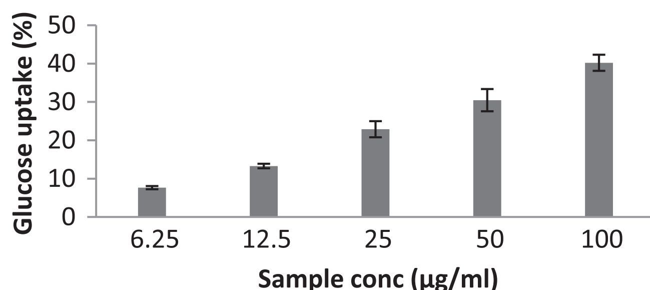

Fig. 3. *Invitro* glucose uptake of Hep G2 cell lines. About 6.25–100 µg sample from the corm of *C. x hortulanum* was loaded into each well. (Error bar: Standard deviation, n = 3).

the glucose uptake was 7.657 ± 0.42% which was increased as 40. 21 ± 2.1% at 100 µg/ml concentration (Fig. 3). The corm extract showed anti-diabetic properties by the inhibitory action in adipogenesis and stimulation of glucose uptake in adipocytes. Results revealed that *C. x hortulanum* corm extract showed significant antidiabetic property and was dose dependent. Recently, Cordero-Herrera et al. (2013) used HepG2 cells to elucidate the antidiabetic property of the plant extract against GLUT-2 transporters. The medicinal plants possess antidiabetic activity and the mechanism of action was elucidated from plants such as *Brassica juncea*, *Combretum micranthum*, *Elephantopus scaber*, *Gymnema sylvestre*, *Smallanthus sonchifolius*, *Swertia punicea* and *Vernonia anthelmintica* (Arumugam et al., 2013). The antidiabetic properties of *Vinca rosea* have been elucidated recently. The ethanolic extract showed potent antihyperglycemic activity in alloxan-induced hyperglycemic rats without much change in body weight. The other vital parameters such as lipid profile, body weight, serum alkaline phosphatase and serum urea level were not changed during experimental trial (Ahmed et al., 2010).

3.4. Antiinflammatory activity of secondary metabolities from the corm of C. x hortulanum

Treatment of cells with corm extract at various concentrations showed a dose-dependent response on the cells as shown in Fig. 4. At 100 µg/ml concentration, inhibitory effect was maximum (45.85 ± 1.8%). Large number of herbal plants was used as folk medicine or traditional medicine against various inflammatory disorders. Most of these anti-inflammatory agents have validated and proved to be potential anti-inflammatory agents. In plants, compounds such as flavonoids, alkaloids, terpenoids, peptides and saponins have been found to be responsible to cure inflammatory disorders (Wen et al., 2004). Ilavarasan et al. (2006) reported the anti-inflammatory activity of alcoholic extract of *Ricinus communis* (Euphorbiaceae) on Wistar albino rats. Many steroidal and non steroidal anti-inflammatory synthetic drugs are widely used in the treatment of acute inflammatory disorders and have side effects such as cardiovascular, renal failure and gastrointestinal damage (Lafrance and Miller, 2009). In medicinal plants the availability of polyphenolics is very important for antiinflammatory activity (Gulcin et al., 2008). In this study, the phenolic content was in the corm of *C. x hortulanum*, could be the reason for its immense antioxidant properties. The bioactive compounds isolated from the roots of *Sanguisorba officinalis* and the isolated

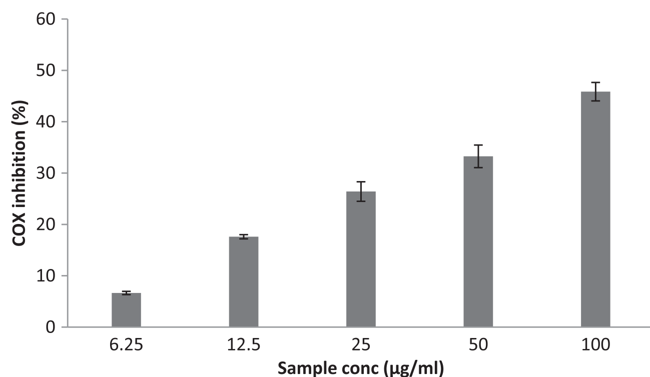

Fig. 4. Anti inflammatory effects of corm extract from *C. x hortulanum*. Murine monocyclic macrophage cell line RAW 264.7 was used for this assay. The cyclooxygenase (COX) inhibition was determined using spectrophotometry method. Samples were loaded into the micro titer plate at increasing concentrations and inhibition (%) was assayed. (Error bar: Standard deviation, n = 3).

rhynchophylline and isorhynchophylline showed potential anti-inflamatory activities (Yuan et al., 2009).

3.5. Hepatoprotective activity properties of secondary metabolites from the corm of C. x hortulanum

In this study, the corm extract of *C. x hortulanum* showed potent hepatoprotective activity. The CCl_4 incorporated cells showed 19. 629 ± 1.5% viability, whereas it was 21.1 ± 1.32% at 6.25 µg corm extract. The cell viability was 49.58 ± 1.91% at 12.5 µg/ml and increased gradually up to 100 µg/ml (Fig. 5). This result was in accordance the observations made previously with other medicinal plants. Yu-Hua et al. (2005) reported the hepatoprotective activity of *Cudrania tricuspidata* in *invitro* in Hep G2 cells derived from human liver and found four isoprenylated xanthones namely, cudraxanthone A, cudraxanthone B and cudraxanthone L. Xinpeng et al. (2011) reported that the root extract of *Piper nigrum* L. showed good hepatoprotective activity against CCl_4 – induced liver injury in animal model. It was revealed that more than 50% of acute liver failure was mainly due to over dose of acetaminophen (39%) and other drugs. Hepatic-cell injury was mainly caused by various antibiotics, carbon tetrachloride (CCl_4), thioacetamide and chemotherapeutic agents (Micheale and Cynthiya, 2006). The hepatoprotective activities of few medicinal plants, namely, *Phyllanthus amarus* (Pramyothin et al., 2007), *Zizyphus Mauritian* (Dahiru et al., 2005), *Cassia tora* (Rajan et al., 2009) and *Allium hirtifolium* (Kazemi et al., 2010).

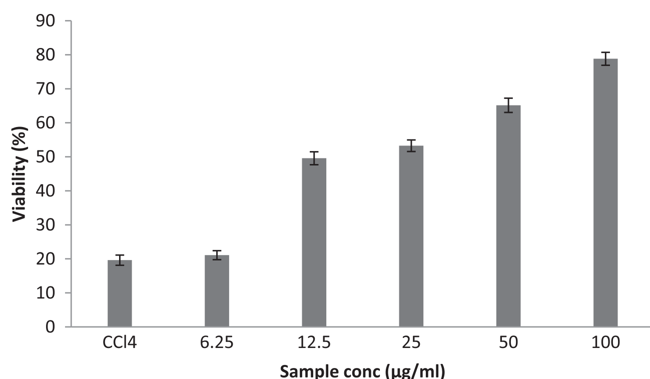

Fig. 5. MTT cytotoxicity assay. Hepatoprotective activity screening was carried out using human liver cells derived Hep G2 cells against cell damage induced by CCl_4. Optical density of the titre plate was read at 540 nm against DMSO blank. The viability of cells increased at high concentrations of secondary metabolites from the corm extract of *C. x hortulanum.* (Error bar: Standard deviation, n = 3).

4. Conclusion

To conclude, *Caladium x hortulanum* corm contains rich diversity of phytochemicals responsible for antimicrobial, antidiabetic, anti-inflammatory and hepatoprotective properties. Hence, the corm of this plant is a good source of drugs to treat or control various diseases. Further investigation should be done to carry out to explore the mechanism of action of potent phytochemical constituents with anti-inflammatory, antidiabetic and hepatoprotective properties.

Declaration of conflict of interest

None declared.

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