

IN-VITRO ANTIOXIDANT ACTIVITY OF *CANNA INDICA* EXTRACTS USING DIFFERENT SOLVENT SYSTEM

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ABSTRACT

Objective: To investigate the antimicrobial and antidiabetic potential of *Canna indica* plant extracts.

Methods: In the present research work, the selected plant, i.e., *C. indica* L. Cannaceae was collected, dried and extracted with different solvents. Different tests were performed for the presence of different phytochemicals. The antimicrobial activity was determined by agar well-diffusion method. The extracts were evaluated for antidiabetic activity using alpha-amylase and alpha-glucosidase enzyme inhibition method.

Results: The findings of this study indicate that *C. indica* extracts contain secondary metabolites which have potent antimicrobial and antidiabetic activities comparable to standard drugs.

Conclusion: This information may help to develop potent bioactive compound(s) in the pharmaceutical industry for the development of drugs.

Keywords: Antimicrobial activity, Antidiabetic activity, *Canna indica*, Phytochemical analysis, Soxhlet extraction.

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INTRODUCTION

The use of plants for treating diseases is as old as the human species. All over the globe, the use of medicinal plants has significantly supported primary health care [1]. From 250 to 500 thousand plant species are estimated to exist on the planet, and only between 1% and 10% are used as food by humans and other animals [2]. Plants synthesize many components, which act as defensive agent, helping to protect them from microbial infection and other diseases. Those compounds are bioactive and can be medicinal, intoxicating or toxic depending on circumstance. Several plants species have been tested for antimicrobial properties, but vast majorities have not yet been adequately evaluated [3]. Various studies have been published, investigating the antifungal and antibacterial activities of plant-derived compounds against a range of pathogens [4-8]. Different substances have been identified in medicinal plants which are believed to be the antimicrobial agent, and these include different forms of alkaloids, diterpenes, saponins, flavonoids, sterols, quinines, different forms of other proteins as well as lipids [9]. Diabetes mellitus is a common and very prevalent disease affecting the citizens of both developed and developing countries. It is estimated that 25% of the world population is affected by this disease [10]. The herbal drugs with antidiabetic activity are yet to be commercially formulated as modern medicines, even though they have been acclaimed for their therapeutic properties in the traditional systems of medicine [11].

Canna Linn. (Cannaceae) is a genus of herbs with rhizomatous rootstocks, distributed in the tropics and subtropics particularly of the western hemisphere. *Canna indica* L. is commonly known as Indian shot or Canna lily. It is extensively used in constructed wetland for removal of organic pollutants, nitrogen, phosphorous, and heavy metals [12-13]. In folkloric medicine, root decoction is used for the treatment of fever, dropsy, and dyspepsia. Seed juice is used to relieve earaches. The leaves of *C. indica* showed antimicrobial activity [14], analgesic activity, and the rhizomes showed a good anthelmintic activity against *Pheretima posthuma* [15]. The flower is said to cure eye diseases and shows antibacterial activity [16]. Flowers contain lutein, β -carotene, and violaxanthin. Its leaves have chemical constituents such as lignin, furfural, and hemicelluloses. While rhizomes have 5,8 - hemicosidine, tetracosane, tricosane [17,18]. The water extract of rhizomes of

C. indica has been reported to have HIV-1 reverse transcriptase inhibitory activity [19] while its essential oil shows antibacterial activity [20]. Methanolic extract of aerial parts of *C. indica* shows antioxidant activity [21]. Anthocyanins and methylated anthocyanin glycosides were also isolated from *C. indica* flowers [22,23]. In this study, we determine the antioxidant activity of *C. indica* extracts which may help to develop potent bioactive compound(s) in the pharmaceutical industry for the development of drugs.

METHODS

Collection and identification of plant material

The fresh aerial part of the plant *C. indica* L. Cannaceae was collected from a local herbal garden in Dehradun. The plant sample was authenticated by Botanical Survey of India, Dehradun with the accession no. 113531. The plant sample was dried in shed at room temperature and crushed to coarse powder.

Preparation of extracts

The dry leaves powder was subjected to successive soxhlet extraction with different solvents in increasing order of polarity (i.e., Petroleum Ether < Chloroform < Ethanol). In this method of soxhlet extraction, a small volume of hot liquid is made to evaporate through a column again and again by heating and subsequent condensation. Thus, the same quantity of menstruum is recycled every time, and complete extraction is achieved with a very small volume of menstruum [24,25].

- Percentage yield of the crude extracts were calculated with the formula:

$$\text{Percentage of yield} = \frac{\text{Weight of extract (gm)}}{\text{Weight of power are taken (gm)}} \times 100$$

Phytochemical analysis of the extracts

Different tests were performed for the presence of different phytochemicals according to Lamaeswari and Ananthi (2012) [26].

Test for carbohydrates

For 2 ml of extract, 2 drops of Molisch's reagent was added and shaken well 2 ml of concentrated H_2SO_4 was added on the sides of the test tube.

A reddish violet ring appeared at the junction of two layers immediately indicated the presence of carbohydrates.

Test for proteins

For 2 ml of protein solution, 1 ml of 40% NaOH solution and 1-2 drops of 1% CuSO₄ solution was added. A violet color indicated the presence of peptide linkage of the molecule.

Test for amino acids

For 2 ml of sample, added 2 ml of ninhydrin reagent and kept in water bath for 20 minutes. The appearance of purple color indicated the presence of amino acids the sample.

Test for steroids

About 2 ml of acetic anhydride was added to 0.5 g of an ethanolic extract of sample with 2 ml of H₂SO₄. The color change from violet to blue or green indicated the presence of steroids.

Test for alkaloids

For the extract, added 1% HCl and 6 drops of Mayer's reagent and Dragendroff's reagent. An organic precipitate indicated the presence of alkaloids in the sample.

Test for flavonoids

About 5 ml of dilute ammonia solution were added to a portion of aqueous filtrate of plant extract followed by addition of concentrated H₂SO₄. A yellow coloration is observed which confirms the presence of flavonoids and it disappears on standing.

Test for tannins

About 5 ml of extract was added to few drops of 1% lead acetate. A yellow precipitate indicated the presence of tannins.

Test for terpenoids

About 5 ml of extract was treated with 2 ml of chloroform and 3 ml of concentrated H₂SO₄ to form a monolayer of reddish brown coloration of the interface was showed to from the positive result for the terpenoids.

Determination of antimicrobial activity

Source of micro-organisms

The organisms used were *Staphylococcus epidermis* MTCC 435, *Micrococcus luteus* MTCC106, *Bacillus subtilis* MTCC 441, *Bacillus cereus* MTCC 645, *Staphylococcus aureus* (clinical), *Escherichia coli* (clinical), *Pseudomonas aeruginosa* MTCC 424, *Salmonella typhimurium* MTCC 733, *Klebsiella pneumoniae* MTCC 109, *Aspergillus flavus*, *Aspergillus niger*, *Fusarium* and *Nigrospora oryzae*. The organisms were obtained from IMTECH, Chandigarh.

Antibacterial assay

The antibacterial activity of different extracts was determined by agar well-diffusion method. The molten Mullar-Hinton agar was added to pre-sterilized plates and 0.1 ml of 12-16 hrs incubated culture of bacterial species were spreaded over the agar plates before spreading cultures was adjusted to MacFarlean constant. Wells were bored into the medium using well puncher syringe. Extract and isolated essential oil were dissolved in 30% DMSO to prepare 100 µl/ml solution. 0.1 ml of each solution was transferred in wells of different plates. The plates were then incubated at 37°C for 24 hrs in BOD incubator. The diameter of the zone of inhibition was measured in millimeter and compared with zone of standard antibiotic solute [27].

Antifungal assay

The antifungal assay was also performed by the well diffusion method. The well diffusion assay was used to screen for antifungal activity

this was accomplished by placing a known amount of the extract in a small well. The wells were punctured on potato dextrose agar growth medium containing a confluent lawn of fungi. The absence of fungal growth around the well containing the extracts, indicate that the plant extracts have antifungal activity against that particular fungus [27].

Determination of antidiabetic activity

The antidiabetic activity of *C. indica* extracts was determined by using alpha-amylase and alpha-glucosidase enzyme inhibition methods.

Inhibition of alpha-amylase enzyme

Starch solution (0.1% w/v) was prepared by stirring 0.1 g of potato starch in 100 ml of 16 mM of sodium acetate buffer. The enzyme solution was prepared by mixing 27.5 mg of alpha-amylase in 100 ml of distilled water. The colorimetric reagent was prepared by mixing sodium potassium tartrate solution and 3, 5 dinitrosalicylic acid solution at 96 mM concentration. Both control and plant extracts separately were added with starch solution and left to react with alpha- amylase solution under alkaline conditions at 25°C. The reaction was measured after 3 minutes. The generation of maltose was quantified by the reduction of 3, 5 dinitrosalicylic acid to 3- amino-5- nitrosalicylic acid. This reaction is detectable at 540 nm [28].

Inhibition of alpha-glucosidase enzyme

The inhibitory activity of alpha-glucosidase enzyme was determined by incubating 1 ml solution of starch substrate (2 % w/v maltose or sucrose) with 0.2 M Tris buffer pH 8.0 and plant extracts separately for 5 minutes at 37°C. The reaction was initiated by adding 1 ml of alpha-glucosidase enzyme (1 U/ml) to it followed by incubation for 40 minutes at 35°C. Then, the reaction was terminated by the addition of 2 ml of 6 N HCl. Then, the intensity of the color was measured at 540 nm [29].

RESULTS

% yield of the extracts

Solvents on the basis of increasing polarity were used for the extraction. It was observed that the highest percentage yield of extract was found in water (9.48%) followed by chloroform (3.67%), petroleum ether (2.52%), and ethanol (1.92%) (Table 1).

Phytochemical analysis of the extracts

The results obtained for qualitative screening of phytochemicals in the extracts are presented in Table 2. In all, more phytochemicals were found in the ethanolic extract. Remarkably steroids are absent in ethanolic extract.

Determination of antimicrobial activity

The antimicrobial activity of extracts from *C. indica* could be attributed to the broad spectrum of bioactive chemical compounds. The study showed remarkable antimicrobial activity. The plant has potent antibacterial activity with zone of inhibition of 21 mm in the case of petroleum ether extract against *M. luteus*, followed by 20 mm in ethanolic extract against *M. luteus*, *S. aureus* and *K. pneumoniae*. *P. aeruginosa* showed minimum activity (11 mm) in the ethanolic extract.

Among the selected fungal strains the highest activity was seen for *A. niger* (16mm) in the ethanolic extract. The water extract do not show any activity against any of the selected microbes (Table 3).

Table 1: % yield of the extracts

S.N	Solvent used	<i>Canna indica</i> (%)
1	Petroleum ether	2.52
2	Chloroform	3.67
3	Ethanol	1.92
4	Water	9.48

C. indica: *Canna indica*

Table 2: Phytochemical analysis of the extracts

Phytochemical present	Plant	Pet ether	CHCl ₃	EtOH	Water	Previous research
Carbohydrates	<i>C. indica</i>	-	-	+	+	+ve in flower [26]
Protein and amino acids	<i>C. indica</i>	-	-	+	+	-ve in flower [26]
Steroids	<i>C. indica</i>	+	+	-	-	
Alkaloids	<i>C. indica</i>	+	+	+	-	+ve in flower [26]
Phenolics and flavonoids	<i>C. indica</i>	-	+	+	-	+ve in flower [26]
Tannins	<i>C. indica</i>	-	-	+	+	
Terpenoids	<i>C. indica</i>	-	-	+	+	+ve in flower [26]

C. indica: *Canna indica*

Table 3: Determination of antimicrobial activity

Bacterial Strain	Petroleum ether	Chloroform	Ethanol	Water	Ampicillin
<i>Micrococcus luteus</i>	21±0.02	-	20±0.02	-	18±0.02
<i>Staphylococcus aureus</i>	20±0.02	-	18±0.02	-	18±0.02
<i>Pseudomonas aeruginosa</i>	13±0.01	-	11±0.01	-	10±0.01
<i>Salmonella typhimurium</i>	-	-	-	-	11±0.01
<i>Klebsiella pneumoniae</i>	20±0.02	19±0.02	19±0.02	-	17±0.02
<i>Escherichia coli</i>	15±0.01	13±0.01	12±0.01	-	12±0.01
<i>Streptococcus mutans</i>	-	-	12±0.01	-	14±0.01
<i>Bacillus cereus</i>	-	-	-	-	12±0.01
Fungal Strains					Ketaconazole
<i>Aspegillus niger</i>	-	12±0.01	16±0.02	-	24±0.02
<i>Aspergillus flavus</i>	-	-	12±0.01	-	21±0.02
<i>Fusarium solani</i>	-	-	14±0.02	-	19±0.02
<i>Nigrospora oryzae</i>	-	-	10±0.01	-	22±0.02

All values shows diameter of zone of inhibition in mm. *A. niger*: *Aspegillus niger*, *A. flavus*: *Aspergillus flavus*, *F. solani*: *Fusarium solani*, *N. oryzae*: *Nigrospora oryzae*

Table 4: Percent reduction in α amylase enzyme activity by extracts and positive control

Extracts	Percent reduction in α amylase enzyme activity (%)
<i>Canna indica</i> petroleum ether extract	73.5
<i>Canna indica</i> chloroformic extract	72.6
<i>Canna indica</i> ethanolic extract	80.0
<i>Canna indica</i> water extract	56.34
Glycinamide (+ve control)	90.56

C. indica: *Canna indica*

Table 5: Percent reduction in α glucosidase enzyme activity by extracts and positive control

Extracts	Percent reduction in α glucosidase enzyme activity (%)
<i>Canna indica</i> petroleum ether extract	75
<i>Canna indica</i> chloroformic extract	73
<i>Canna indica</i> ethanolic extract	82
<i>Canna indica</i> water extract	64
Glycinamide (+ve control)	92

C. indica: *Canna indica*

Determination of antidiabetic activity

The antidiabetic activity of the *C. indica* extracts was assayed by reduction in α amylase and α glucosidase enzyme activity. In α amylase enzyme activity, the maximum reduction was found in the ethanolic extract (80.0%) followed by petroleum ether extract (73.5%) and chloroform extract (72.6%). The minimum reduction was found in water extract (56.34%) (Table 4). In α glucosidase enzyme activity, the maximum reduction was found in ethanolic extract (82.0%) followed by petroleum ether extract (75.0%) and chloroform extract (73.0%). The minimum reduction was found in water extract (64.0%) (Table 5). Both the activities were compared with the standard antidiabetic agent glycinamide as +ve control.

DISCUSSION

According to Tiwari *et al.* (2011), the factors affecting the choice of solvent are the quantity of phytochemicals to be extracted, rate of extraction, diversity of different compounds extracted, diversity of inhibitory compounds extracted, ease of subsequent handling of the extracts, toxicity of the solvent in the bioassay process, potential health hazard of the extractant [30]. According to Gaur *et al.* (2014), the extract shows good antibacterial activity against *S. aureus*, *B. subtilis* and mild active against *E. coli* [31]. According to George (2014), the antimicrobial activity of the oil showed significant inhibitory activity against the human pathogenic bacteria and no activity was observed against the fungi *A. niger* and *Fusarium oxysporum* [32]. Purintrapiban *et al.* (2006) also reported the effects of *C. indica* extracts, which has been used as a traditional medicine for treating diabetes on glucose transport activity, which was evaluated in cultured L8 muscle cells. The aqueous extract of *C. indica* root (CI) at doses of 0.1-0.5 mg/ml, which contains total phenolic compounds equivalent to 6-30m g of catechin caused a dose and time-dependent induction of 2-deoxy-[3H] glucose (2-DG) uptake activity [33].

CONCLUSION

C. indica (L.) is a well-known plant with less known scientific data. The folkloric medicinal value imparts tremendous value to this herb. The qualitative analysis revealed the presence of the phytochemical such as carbohydrates, proteins, amino acids, steroids, alkaloids, phenolics, flavonoids, tannins, and terpenoids. The present work shows that *C. indica* is a medicinal plant with anti-microbial and anti-diabetic activities which could be utilized in several medicinal applications because of its effectiveness. Therefore, there is wide scope for research in the direction of more medicinal activities of plant and to evaluate the pharmacological actions of the same incoming future.

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