Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(3): 93-97
Received: 07-03-2017
Accepted: 08-04-2017

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Phytochemical profiling, antibacterial activity and antioxidant potential of *Cascabela thevetia* (L.) whole plant extracts

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Abstract
Plants are the excellent source of various herbal medicines useful in the treatment of various human diseases. Natural products from plants are traditionally used for the treatment of antibacterial and antioxidant mainly in developing countries where the resources are limited and access to modern treatment is a problem. In the present study, preliminary phytochemical profiling analysis was carried out on the whole plant (leaves, stem, root and flower) extract of chloroform, methanol and ethanol extract of *Cascabela thevetia* for identification of phytocompounds in the conformation and Separation of bioactive fractions of methanol whole plant extract of *C. thevetia* by TLC**.** antibacterial activity against the bacterial strains of *Pseudomonas aeroginosa* (10.4, 4) *Eschirechia coli* (7.9 mm), *Klebsiella pneumonia* (7 mm), *Proteus vulgaris* (9.7 mm), *Staphylococcus aureus* (9.4 mm) methanol plant extract the best activity to compare with other extracts respectively. Methanol extracts of whole plant showed remarkable antioxidant activity 0.912±0.0017 and IC_{50} value 60.20 % at100 µg/µl respectively.

Keywords: *Cascabela thevetia,* TLC, antibacterial and DPPH Assay

Introduction
The plant based, traditional medicine systems continues to play an essential role with about 80% of the World's inhabitants relying mainly on traditional medicines for their primary health care [1].
"Mother Nature" remains the unchallenged source for new drug discovery throughout the span of human history. Indian medicinal plants are widely used by all people throughout the World either as a folk remedy or in different indigenous systems of medicine or in the pharmaceutical preparations of modern medicine. Medicinal plants and their products continue to be used as a common practice in traditional medicine for the cure of many diseases including cancer. Drugs from plants are easily available, efficient and less expensive and could be handled easily; yet they have negligible side effects. Their role is two-fold for the development of new drugs: either they serve as a base material for the development of drugs or as a phytomedicines to treat many diseases [2].
Medicinal plants are abundant sources of antimicrobial molecules. Wide ranges of medicinal plants extract are used to treat several infections as they have potential antimicrobial activity. Some of these bioactive molecules are screened and traded in the markets as raw material for many herbal industries [3]. Phytochemicals are chemical compounds formed during the plants normal metabolic processes, which are often referred to as "secondary metabolites". There are several secondary metabolites including alkaloids, coumarins, flavonoids, glycosides, phenols, tannins, terpenes and triterpenoids [4-5].
Antioxidant is any substance when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of substrate. The term 'oxidizable substrate' includes almost everything found in the living cells including proteins, lipids, DNA and carbohydrates [6].
All parts of the *C. thevetia* plant are toxic to most vertebrates as they contain cardiac glycosides. Many cases of intentional and accidental poisoning of humans are known. The main toxinsarethe cardenolides called thevetin A and thevetin B; othersinclude peruvoside, neriifolin, thevetoxin and ruvoside [7].

Material and methods
The plants *Cascabela thevetia* (L.) Lipoid, were collected near the Marina Beach (seashore), Chennai, Tamil Nadu. The plant were authentically identified by Prof. P. Jayaraman, Institute of Herbal Botany, Plant anatomy research centre,

West Tambaram, Chennai, Tamil Nadu, India and the voucher specimen No: PARC/2016/3284.

Preparation of extracts

The plant material (500 g) was shade dried and ground to powder. Three portions (about 10 g each) of the same were taken separately in 100 mL each of chloroform, methanol and water and refluxed for 2 h to obtain the respective extracts. After filtration, the non-polar solvents of the chloroform and methanol extracts were removed in a rotary evaporator. The water extract was filtered through Whatman filter paper No. 1, concentrated and freeze-dried in a lyophiliser. The yield (% w/w) of the crude extracts obtained from chloroform, methanol and water were recorded and named as CTC, CTM and CTW, respectively.

Human pathogenic bacteria species

The human pathogenic bacteria such as *Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas auroginosa, Escherichia coli, and Proteus vulgaris* were obtained from the Department of Microbiology, Presidency College, and Chennai - 600005.

Preparation of sterile disc

Each sterile disc (6mm) was incorporated individually with 2, 4, 6, 8, 10 and 12 µg/µl of the extracts using micropipette. Norflaxin was used as standard (10 µg/µl) and the precautions were taken to prevent the flow of the solvent extract from the disc to the outer surface. The condensed extracts were applied in small quantities on discs and they were allowed to dry in air. After sometimes another doses of extracts were applied on discs. Then they were stored at 4ºC.

Standardization of crude whole plant extracts against human pathogens by disc diffusion method

The 25 ml of sterilized Muller Hinton Agar was poured into sterile petriplates, after solidification, 100µl of fresh culture of human pathogens were swabbed on the respective plates. The discs were kept over the agar plates using sterile forceps at various concentrations (2, 4, 6, 8, 10 and 12µg/µl) and the plates were incubated for 24 hours at 37ºC. After incubation the diameter of inhibitory zones formed around each discs were measured (mm) recorded.

Phytochemical Analysis

Cascabela thevetia (L.) Lippoid the whole plant extracts were analysed for the presence of the following secondary metabolite phyto constituents such as tannins, saponin, flavonoids, alkaloids, quinones, glycosides, terpenoids, triterpenoids, phenols, cumarins, acids, cyanins, cardiac glycosides, proteins and carbohydrates using modified and standard protocols [8-9].

Thin Layer Chromatography

The TLC was performed on precoated 20×20 cm and 0.25 mm thick plates. The plates were prepared by using silica gel G for TLC, were left overnight for air drying. These plates were activated by hot air oven at 100 ºC for 1hr.Cold alcoholic extract was plotted on TLC plates. The plates were dried and developed in suitable solvents for rapid screening chloroform / methanol in the ratio 5:1. The plates were run in the above solvent systems and dried at room temperature. Derivatisation of TLC plates was done by iodine vapor stain. Different bands were observed and corresponding *Rf* values are determined. *Rf* value of each spot was calculated as:

***Rf* = Distance travelled by the solute / Distance travelled by the solvent**

DPPH Assay

The antioxidant potential of the extracts was determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity by the modified method of [10].

Results and Discussion

Antibacterial activity

The methanol, chloroform and aqueous extracts of *C. thevetia* were evaluated for activity against medically important bacterial strains such as *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeroginosa* and *Proteus vulgaris,* respectively. These five different bacterial species have also been tested with commercially available antibiotics Norflaxin. The results were showed fig. 2, 3 and 4 (Bacterial) maximum zone of inhibition was observed in the methanol whole plant extract of C. *thevetia* when compared to the other extracts of chloroform and aqueous. Results indicated that the naturally occurring alkaloids and their synthetic derivatives have analgesic, antisplasmodic and bactericidal activities [5]. Each of this group of compounds has been reported to possess antimicrobial activity [11-13] and reported exert their effects by affecting the cell membrane integrity of the bacteria [14-17].

Phytochemical analysis

Cascabela thevetia whole plant showed the strongly presence for carbohydrates, saponins, alkaloids and terpenoids in aqueous extract and where as chloroform extract showed strongly presence for carbohydrates, saponins and methanol showed strongly presence for carbohydrares, tannins, saponins, alkaloids, phenols, cardiaglycosides and steroids as shown in the table 1. These secondary metabolites contribute significantly towards the biological activities of medicinal plants such as hypoglycemia antidiabetic, antioxidants, antimicrobial, anti-inflammatory, anticacinogenic, antimalavial and antilepory activities etc [18].

Thin Layer chromatography

From the above preliminary phytochemical studies (Qualitative chemical test) the strong presence of *C. thevetia* whole plant methanol extract alone were taken for the further study of thin layer chromatography. Thin layer chromatography (fig.1) shows the presence of compounds in the selected medicinal plant leaves. The retention factor (R_f) of whole plant extracts are shown in table. 2. Methanolic extracts gives an impressive result that directly towards the presence of number of phytochemicals. TLC methods are best choice for the identification of secondary metabolites present in the plants. Here the Different R_f-values indicate the presence of different nature of phytoconsituents in single extracts. Different R_f-value of the compounds also reflects on idea about their polarity. More or less same finding was suggested by [19] with present study this information will help in selection of appropriate solvent system for further separation of compounds from these plant extract [20].

Antioxidant assay

Free radicals induce lipid peroxidation in polyunsaturated lipid rich areas like brain and [21] peroxidation, which involves a series of free radical mediated chain reaction processes, is also associated with several types of biological damage. Therefore much attention has been focused on the use of natural antioxidants to protect from damage due to free

radicals. In the present study, the methanol whole plant extract of *C. thevetia* alone showed 50% inhibition (IC_{50}) at 60.1µg/µl when compared to other extracts as shown in the table-3. The data obtained reveal that the activity of *C. thevetia* in DPPH radical activities were found to possess higher antioxidant activity when compared to other extracts.

DPPH assay has been extensively used for screening antioxidant activity because it can accommodate many samples in a short period and is sensitive enough to detect active ingredients at low concentration [22]. When DPPH radicals encounter a proton donating substance such as an antioxidant, it would be scavenged and the absorbance is reduced. Thus, the DPPH radicals were widely used to investigate the scavenging activity of some natural compounds.

Table 1: Qualitative phytochemical analysis of *C. thevetia* whole plant extracts

S. No	Secondary metabolites	Aqueous	Chloroform	Methanol
1	Carbohydrate	+++	+++	+++
2	Tannins	+	++	+++
3	Saponins	+++	+++	+++
4	Flavonoids	-	-	+
5	Alkaloids	+++	+	+++
6	Anthocyanin	-	-	+
7	Quinones	+	+	++
8	Glycosides	-	-	+
9	Cardiac glycosides	-	-	+++
10	Terpenoids	+++	++	+++
11	Triterpenoids	+	+	+++
12	Phenols	++	++	+++
13	Coumarins	++	+	++
14	Acids	+	-	-
15	Protein	+	-	+
16	Steroids	++	-	+++

+++ Strong presence ++ positive + present - absent

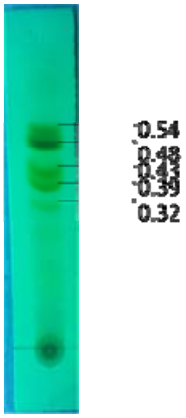

Fig 1: Thin Layer Chromatography of methanol whole plant extract of *C. thevetia.*

Table 2: Thin Layer Chromatography of methanol whole plant extract of *C. thevetia*

Methanol extract	R_f value	Compounds
Band -1	0.54	Alkaloids
Band- 2	0.48	Phenol
Band- 3	0.43	Steroids
Band- 4	0.39	Triterpenoids
Band -5	0.32	Saponins

Table 3: DPPH potential of whole plant extracts of *Cascabela thevetia*

Extracts	Mean ±S.E					IC_{50} values
	20 µg/µl	40 µg/µl	60 µg/µl	80 µg/µl	100 µg/µl	
Aqueous	2.17±0.0009	2.051±0.0006	1.778±0.0006	1.251±0.0004	1.132±0.0009	60.25
Chloroform	0.883±0.0014	0.804±0.0007	0.748±0.0018	0.664±0.0005	0.627±0.0011	60.01
Methanol	2.107±0.0002	1.885±0.0002	1.635±0.0016	1.219±0.0016	0.912±0.0017	60.20

S.E - Standard Error

IC_{50} Value - Minimal inhibitory Concentration

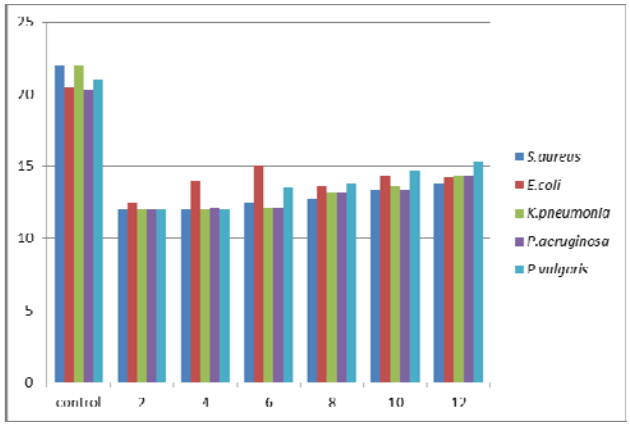

Fig.2: Antibacterial activity of *C. thevetia* whole plant methanol extract

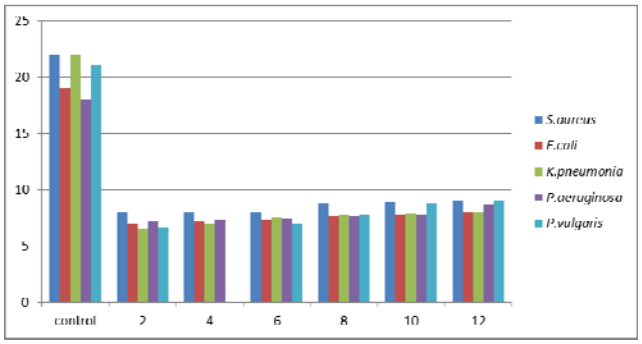

Fig.3: Antibacterial activity of *C. thevetia* whole plant chloroform extract

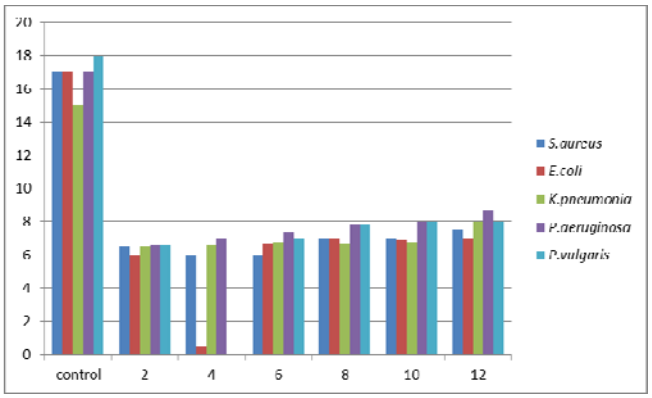

Fig.4: Antibacterial activity of *C. thevetia* whole plant aqueous extract

Conclusions

In the present study the methanol whole plant extract of *C. thevetia* has displayed good antibacterial activity against gram positive and gram negative is playing its potential in the development of new phytopharmaceuticals. As these drugs are plant based, they can be considered safe for human. However, since the experiment conducted was based on crude extract further studies are needed in this direction.

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