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Research Article

TLC ANALYSIS AND ANTIBACTERIAL ACTIVITY OF *CANNA INDICA* L. FLOWERS

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Abstract

The present research work discusses the TLC chromatograms constituted different colored phytochemical compound with different Rf values were determined. Two different solvents (Methanol, Ethanol) and distilled water were used to extract the bioactive compounds from the flowers of *Canna indica* to screen. The antibacterial activity selected human clinical pathogens by agar well diffusion method. The maximum antibacterial activities were observed in phenolic compound other than saponin, sterol, flavonoid and tannin. FT-IR and UV analysis to detect the functional group and confirmed the availability of antibacterial phenol derivative compounds.

Keywords: Antibacterial activity, FT-IR, UV and TLC Analysis.

Introduction

Plants have been used to treat or prevent illness since before recorded history. The sacred Vedas dating back between 3500 B.C and 800 B.C give many references of medicinal plants. One of the remotest works in traditional herbal medicine is "*Virikshayurveda*", compiled even before the beginning of Christian era. "*Rig Veda*", one of the oldest available literatures written around 2000 B.C. mentions the use of Cinnamon (*Cinnamomum verum*), Ginger (*Zingiber officinale*), Sandalwood (*Santalum album*) etc. not only in religious ceremonies but also in medical preparation (Bentley and Trimen, 1980). Plants and plant-based medicaments are the bases of many of the modern pharmaceuticals were used today for our various ailments (Abraham, 1981).

Canna indica is a perennial growing to 1.5 m (5ft) by 0.6 m (2ft). It is hardy to zone 8 and is frost tender. It is in flower from August to October and the seeds ripen in October. The

flowers are hermaphrodite (have both male and female organs). The plant prefers light (sandy), medium (loamy) and heavy (clay) soils and requires well-drained soil. The plant prefers acid, neutral and basic (alkaline) soils. It cannot grow in the shade. It requires moist soil.

The plant is used in the treatment of women's complaints. A decoction of the root with fermented rice is used in the treatment of gonorrhoea and amenorrhoea. The plant is also considered to be demulcent, diaphoretic and diuretic.

Thin layer chromatography (TLC) is a chromatography technique used to separate mixtures. Thin layer chromatography is performed on a sheet of glass, plastic, or aluminum foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminum oxide, or cellulose (blotter paper). This layer of adsorbent is known as the stationary phase. After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action. The separation process is easy to follow; for example, the separation of colored compounds and the distortion of chromatographic zones. Several samples can be separated in parallel and two-dimensional separations are easy to perform. Specific and sensitive color reagents can be used to detect separated spots.

Antibacterial Activity

An antibacterial is a substance that kills or inhibits the growth of bacteria. Antibacterial drugs either kill microbes (Bactericidal) or prevent the growth of microbes (micro biostatic). Disinfectants are antibacterial substances used on non-living objects or outside the body.

Bacterial Characterization

Staphylococcus aureus

Staphylococcus aureus is a facultatively anaerobic, gram positive coccus which appears as grape like clusters when viewed through a microscope and has large, round, golden – yellow colonies, often with hemolysis , when grown on blood agar plates

Bacillus subtilis

B. subtilis known also as the hasy bacillus or grass bacillus is a gram- positive, Catalase – positive bacterium commonly found in soil. *B. subtilis* is rod – shaped and has the ability to from a tough protective endospore *B.subtilis* is a pathogen. It may contaminate food but rarely causes food poisoning..

Escherichia coil

E.coli is gram – negative, facultative anaerobic and non – sporulating. Cells are typically rod shaped about 2 micrometers (µm) long and 0.5µm in diameters *E.coli* uses mixed – acid fermentation in anerobic condition, producing lactase, succinate ,ethanol and carbon dioxide .

Klebsiella pneumonia

K. pneumoniae is a gram negative, non – motile, encapsulated lactose fermenting facultative anaerobic , rod shaped bacterium found in the normal flora of the mouth skin and interfines. In some parts of world, *K.pneumoniae* is an important cause of communities acquired pneumonia in elder persons.

Salmonella typhi

Salmonella is a genus of rod-shaped, Gram-negative,non-spore-forming, predominantly motile enterobacteria with diameters around 0.7 to 1.5 µm, lengths from 2 to 5 µm and flagella which grade in all directions (i.e. peritrichous). *Salmonella* is closely related to the *Escherichia* genus and are found worldwide in cold- and warm-blooded animals (including humans), and in the environment. They cause illnesses like typhoid fever, paratyphoid fever, and food borne illness.

Materials and Method

Sample Collection: The Plant species namely *Canna indica* L. flower were collected in Padappaikkadu and around Thanjavur (Dt), Tamil Nadu. The collected samples were carefully stored in polythene bags and used for the present study.

Sterilization of Plant Materials: The disease free and fresh plants were selected for this investigation. About 2gm fresh and healthy flowers were taken for various solvent such as Ethanol, Methanol and Distilled Water. Then, surface

sterilized with 0.1% mercuric chloride and alcohol from few seconds. Again the plant materials were washed thoroughly with distilled water (Three times).

Preparation of Flower Extracts: 2 grams of sterilized flower were kept in the 10 ml organic solvents such as ethanol, methanol and distilled water. Then these are grind with the help of mortar and pestle. The grind plant material was subjected to centrifugation, for 10-15min (at 10,000rpm). The supernatant was collected and stored for further purposes.

Isolation of phytocompounds from *Canna indica* L. flower

Quanlitative phytocompounds screening was done by TLC (Wagner, 1995).

TLC (Thin Layer Chromatography*)*

The general principle was similar to that of column chromatography i.e. adsorption chromatography. In this adsorption process, the solution completes with the solvent for the surface sites of the adsorbent. Depending on the distribution co - efficients, the compounds used to distributes on the surface of the adsorbent. The adsorbent normally used was an agent such as calcium sulfate with facilitates the holding of the adsorbent to the glass plates.

Purification of phytochemical compounds

TLC using various solvent system to separate phytochemical compounds in the ethanolic and methanolic extract in to visible fractions with retention factor value. The fraction plus the origin were purified from the developed duplicate plate by collecting the silica placing it into a polypropyline test tube and dissolving the fraction in chloroform : methanol (1:1). Centrifuged to remove silica partials and the supernatant was collected used for further antibacterial activity (Reynolds and Dweck, 1999).

Microbial Strains: The human pathogenic bacteria species were collected from the Microbial Germ Plasm Culture Collection Unit (MGPCCU) Sri Gowri Biotech research Academy, nagai Road, Thanjavur.

Antibacterial activity of phytocompounds: Sensitivity of five different bacterial strains to various phytocompounds extracts was measured in turns of zone of inhibition using agar well diffusion assay. The zone of inhibition was calculated by measuring the diameters of the inhibition zone around the well.

Antibiotic sensitivity test on microbes (Positive control)

The antibiotic sensitivity test using Standard Antibiotics (Kanamycin, Chloramphenical and Tetracycline for bacteria) were analysed (Bauer *et al*., 1996). The results were observed and the diameter of the inhibition zone was measured around the isolates.

FT - IR (Fourier Transform – Infra Red Spectroscopy)

The functional groups of phytocompounds were carried out of FT-IR (Lau, 1998).Fourier transform infra red spectroscopy (FT - IR) is a measurement technique for collecting infra red spectra. Instead of recording the amount of energy absorbed when the infra-red light is varied, the IR light is guided through an interferometer. FT-IR spectrometer is cheaper than conventional spectrometers because building an inferometer is easier than the fabrication of a monochrometer. In addition measurement of a signal spectrum is faster for the FT-IR technique because the information at all frequencies is collected simultaneously. Virtually all modern infra red spectrometer are FT-IR instruments.

UV (Ultraviolet visible spectroscopy)

The fractional sample was dissolved in acetone nitrate and then detected its UV absorbance values with Lambda 35 Ultraviolet scanner.

Results and Discussion

TLC Analysis

The successive extracts of *Canna Indica* L. flower have revealed the presence of Sterol, Phenol, Flavonoid, Saponin and Tannin. The results are presented in the **Table 1 and Plate 1.** In the present study Thin Layer Chromatography (TLC) has been conducted for the separation of different component and Rf value of developed spots in different solvent system have been noted. The Rf value can be used as a tool for the standardization of compound.

Thin Layer Chromatography (TLC) is an option that has distinct advantages over several other chromatographic methods such as its simplicity, low cost, high sample capacity and rapid availability of results. It is unique due to the fact that it presents the result as an easy-to-interpret image or chemical fingerprint (Marston, 2007).

Antibacterial activity

Antibacterial activity of phytocompounds separation from Sterol, Flavonoids, Saponin, Phenol and Tannin were tested against *Bacillus subtilis, E.coli, Staphyllococcus aureus, Klebsilla pneumoniae* and *Salmonella typhi.* The results are tabulated in the **Table 2.**

The sterol compound was exhibited maximum zone of inhibition in *Salmonella typhi* (12mm) and *Staphylococcus aureus* (8mm). The minimum zone of inhibition was observed in *E.coli* (7mm) , *Klebsiella pneumoniae* (7mm) and *Bacillus Subtilis* (7mm).

The flavonoids compound showed moderate zone of inhibition in *Klebsiella pneumoniae* (6mm) and *E.coli* (6mm). The minimum zone of inhibition was observed in *Staphylococcus aureus* (3mm) and *Bacillus subtilis* (5mm).

The saponins compound showed maximum zone of inhibition against *Staphylococcus aureus* (10mm). The moderate zone of inhibition (5mm) in *E.coli, Salmonella typhi* and *Klebsiella pneumoniae.* The minimum zone of inhibition was observed in *Bacillus subtilis* (3mm).

The phenols compounds showed maximum zone of inhibition (12mm) in *E.coli* and *Salmonella typhi* the moderate zone of inhibition against *Klebsiella pneumoniae* (10mm) and *Bacillus subtilis* (10mm) . The minimum zone of inhibition was observed in *Staphylococcus aureus* (4mm).

The Tannins compounds showed maximum zone of inhibition (6mm) in *Staphyllococcus aureus.* There is no inhibitory activity against tested *E.coli, Klebsiella pneumoniae, Salmonella typhi* and *Bacillus subtilis.*

Antibacterial activity of phytocompound separation from Sterols, Flavonoids, Saponins, Phenols and Tannins were tested against *Bacillus subtilis, E.coli, Staphyllococcus aureus, Klebsilla pneumoniae* and *Salmonella typhi.* The antimicrobial activity have been screened because of their great medicinal relevance with the recent years, infections have increased to a great extent and resistant against antibiotics, becomes an ever increasing therapeutic problem (Venkatesan, 2010).

Antibiotic Sensitivity Test

The antibiotic sensitivity test using standard antibiotic test such as Tetracycline, Chloramphenical and Kanamycin were tested against bacterial were tested against bacterial organisms studied. The results of antibiotic sensitivity test presented in the **Table 3.**

The standard antibiotic Chloramphenical showed maximum zone of inhibition against *Bacillus subtilis* (15mm), *Staphyllococcus aureus* (12mm) and *Klebsiella pneumoniae* (13mm).The minimum zone of inhibition was observed in *E.coli* (10mm) and *Salmonella typhi* (2mm).

The standard antibiotic Tetracycline was exhibited maximum zone of inhibition against *Bacillus subtilis* (17mm) and *Staphyllococcus aureus* (9mm). The minimum zone of inhibition was observed in *E.coli* (10mm) and *Klebsiella Pneumoniae* (12mm) .There is no inhibitory activity against tested *Salmonella typhi*.

The standard antibiotic Kanamycin showed maximum zone of inhibition against *Bacillus subtilis* (17mm) and *E.coli* (12mm).The moderate zone of inhibition (10mm) in *Staphyllococcus aureus*. The minimum zone of inhibition was observed in *Salmonella typhi* (2mm). There is no inhibitory activity against tested *Klebsiella pneumonia.*

FT-IR Analysis

The FT-IR analysis showed the functional groups of purified phenols as strong bands at 3754.42, 3439.42, 2896.09, 2778.66, 2680.62, 2406.61, 2282.00, 1642.09, 1087.25 and 946.57 cm^{-1} were showed. The different functional groups of purified phenols were showed as Amide group, Amine group, chelating compound CO-H stretching vibration free O-H, C-H alkaline, C-H stretching vibration two band, Amine N-H stretching , Hydroxyl , stretching bond, O-H stretching vibrations free, unsaturated Nitrogen compound , O-NO_2, Nitrates, Sulfur compound, C=S stretching vibrations and Halogen compounds C-X stretching vibrations C-Cl.The results are tabulated in the **Table 4, Fig 1.**

The stem bark part of the plant, *Helicteres isora* was analysed phytochemically and a compound was isolated the petroleum ether extract. The compound was characterized by FT-IR analysis was found to be a β-Sitosterol (Badgujar and Jain, 2009)

Non linear optical property of the phenol compound has been tested by Kurtz powder technique. Its optical behavior was examined by ultraviolet-visible spectrum instrument model Lamda35 and found the crystal is transparent in the region between 400-1100 nm. UV spectra was recorded from the purified phenol as the function of reaction. The bond were recorded at studiedly increase the intensity at the time of function of reaction without any shift in the peak wavelength. The results are tabulated in the **Table 5, Fig 2.** The UV spectrum of PHI-E showed at λmax 251,265,290 and 315 (Data). (Badgujar *et al.,* 2009)**.**

Table-1: Separation of Phytocompounds by Thin Layer Chromatography (TLC).

S.No	Name of the compounds	Reagents Used	Observation	Results	Rf value
1	Flavonoids	-	Yellow color spot	+	0.89
2	Saponins	Iodine vapour	Yellow color spot	+	0.92
3	Sterols	Folin ciocalteu's reagent	Blue color spot	+	0.92
4	Phenols	Folin ciocalteu's reagent	Blue color spot	+	0.66
5	Tannins	10% $FeCl_2$ in ethanol	Blue color spot	+	0.88

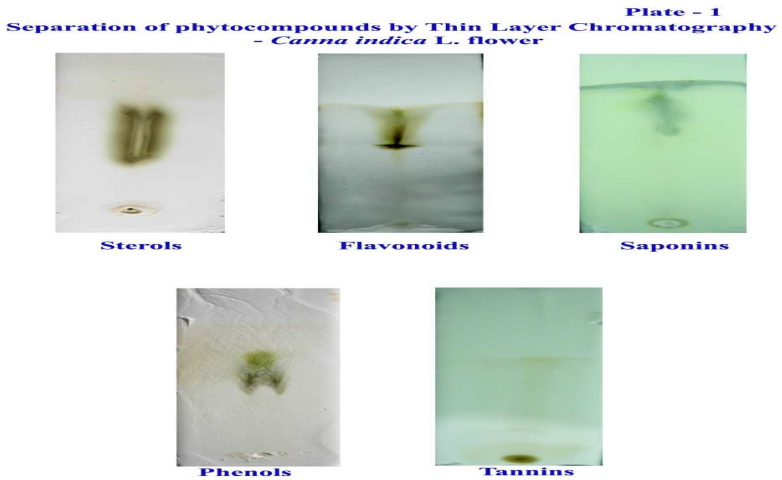


Table-2: Antibacterial activity of Phytocompounds.

S. No.	Test organisms (Bacterial pathogens)	Flavonoids (200µl)	Phenols (200µl)	Saponins (200µl)	Sterols (200µl)	Tannins (200µl)
		Zone of inhibition (diameter in mm)				
1.	*Bacillus subtilis*	5	10	3	7	-
2.	*E.coli*	6	12	5	7	-
3.	*Klebseilla pneumoniae*	6	11	5	7	-
4.	*Salmonella typhi*	8	12	5	12	-
5.	*Staphylococcus aureus*	3	4	10	8	6

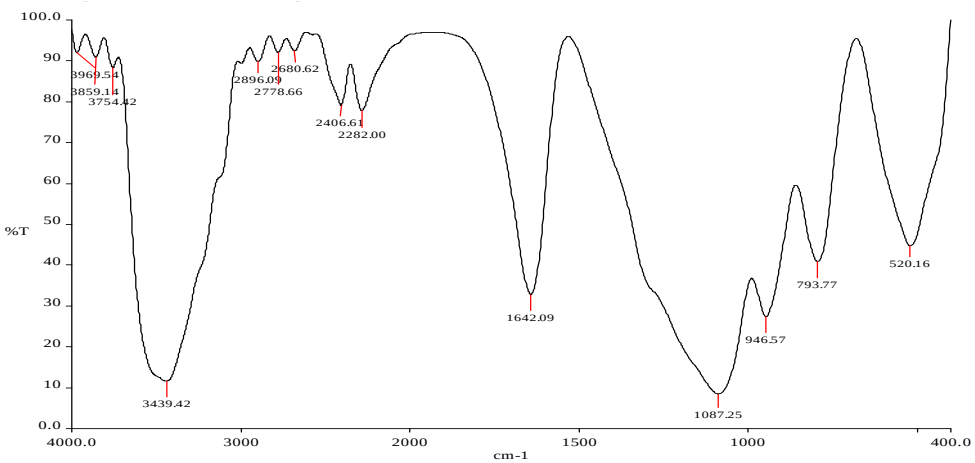
Table-3: Antibiotic Sensitivity test on bacteria (Positive control).

S.No.	Test Organisms (Bacterial pathogens)	Chloramphenical (200µg)	Kanamycin (200µg)	Tetracycline (200µg)
		Inhibition Zone of growth (Diameter in mm)		
1.	*Bacillus subtillis*	15	17	17
2.	*E.coli*	10	12	10
3.	*Klebseilla pneumoniae*	13	-	12
4.	*Salmonella typhi*	2	2	-
5.	*Staphylococcus aureus*	12	10	9

Table 4: Detection of various functional groups using FT-IR.

S. No.	Group Frequency cm^{-1} of the sample	Functional Group Assignment
1.	3754.42	Amides groups, Secondary bonded
2.	3439.42	Amine, Secondary free; One bond
3.	2896.09	Chelating compound CO-H stretching vibration free O-H
4.	2778.66	C-H alkaline, C-H stretching vibration two band (Aldehyde)
5.	2680.62	Amine N-H stretching
6.	2406.61	Hydroxyl, Stretching bond
7.	2282.00	O-H stretching vibrations free
8.	1642.09	Unsaturated Nitrogen compound, O-NO_2, Nitrates
9.	1087.25	Sulfur compound, C=S stretching vibrations
10.	946.57	Halogen compounds C-X stretching vibrations C-Cl

Fig 1: Detection of various functional groups using FT-IR



TNJ-L.pk

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2778.66 92.02 2680.62 92.49 2406.61 79.17 2282.00 77.82 1642.09 32.86
1087.25 8.49 946.57 27.35 793.77 40.85 520.16 44.72

Table 5: UV Spectrum

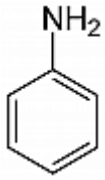
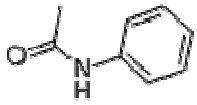
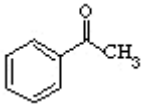
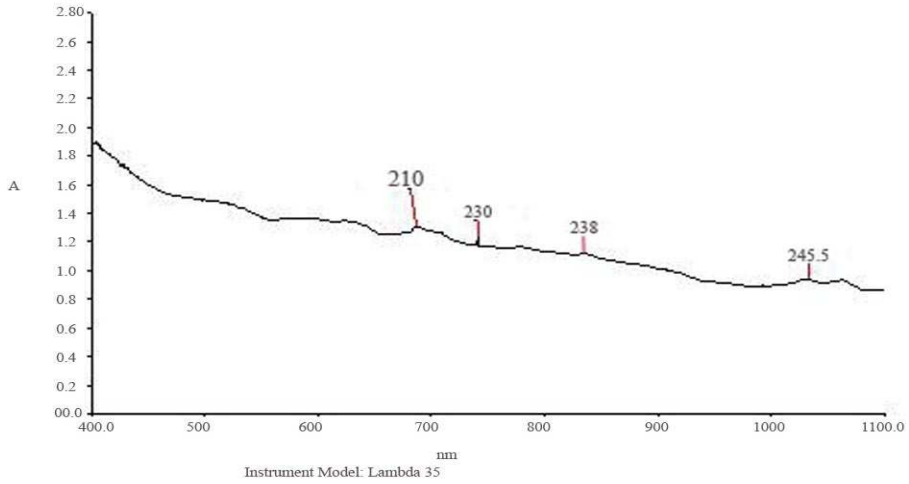
S.NO	λmax	Compound Name	Solvent	Structure
1.	210	Bromo benzene	Methanol	
2.	230	Aniline	Methanol	
3.	238	Acetanilide	Methanol	
4.	245.5	Methylphenyl ketone	Methanol	

Fig 2: UV Spectrum



Conclusion

It has been concluded from these studies it is highly essential for raw drugs on plant parts used for the preparation of compound formulation drugs. The periodic assessment is essential for quality assurance and safe use of herbal drugs.

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