https://doi.org/10.33472/AFJBS.6 9.2024.3857-3868

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Isolation of active constituent from (MECD) Methanolic extracts of *Cordia Dichotoma Forst* leaves by Column Chromatography

Mr.Rahul Batusing Lovhare1*, Dr.Priyanka Soni 2*, Dr.Rajesh Arjun Ahirrao 3*

1. Research Scholar, Faculty of Pharmacy, Mandsaur University, Mandsaur (M.P).
Email:aishraha83@gmail.com
2. Professor, Faculty of Pharmacy, Mandsaur University, Mandsaur (M.P).
Email: drpriyanka.soni@meu.edu.in
3. Principal, P.G. College of Pharmaceutical Sciences, Post. Chaupale Tal. & Dist. Nandurbar. (MH).
Email: rajahirrao1@gmail.com

Volume 6, Issue 9, 2024

Received: 09 March 2024

Accepted: 10 April 2024

Published: 20 May 2024
doi:10.33472/AFJBS.6.9.2024.3857-3868

Abstract

Cordia dichotoma Forst a plant species commonly found in the Western Ghats of India, has been traditionally used in various folk remedies to treat various ailments, including inflammation, fever, and skin diseases. The methanolic extract of *Cordia dichotoma Forst* leaves has been reported to exhibit significant anticancer, antioxidant, antimicrobial activities. However, the isolation and characterization of the active constituents responsible for these activities are still limited. In this study, we report the isolation and characterization of an active constituent from the methanolic extracts of *Cordia dichotoma Forst* leaves using column chromatography techniques[1].

The methanolic extract was subjected to column chromatography using a combination of silica gel and Sephadex LH-20 columns. The resulting fractions were analyzed using thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC) to identify the active constituents. The results showed that the active constituent was isolated as a yellowish-brown solid with a melting point of 120-125°C. The compound was characterized using spectroscopic techniques, including UV-Vis, IR, and NMR spectroscopy[2]. The results revealed that the isolated compound was a phenolic compound, specifically a flavonoid, identified as 7-hydroxy-2-(4-nitrophenyl)-4H-chromen-4-one. The findings of this study provide evidence for the traditional use of *Cordia dichotoma Forst* leaves in folk remedies and highlight its potential as a natural source of anticancer, antioxidant, antimicrobial agents.

The isolation and characterization of 7-hydroxy-2-(4-nitrophenyl)-4H-chromen-4-one provide valuable insights into the chemical composition of the plant and its potential applications in the development of new therapeutic agents[3].

Keywords: Cordia Dichotoma,7-hydroxy-2-(4-nitrophenyl)-4H-chromen-4-one, HPLC.

Introduction

Cordia dichotoma Forst (also known as Indian cherry) belonging to the family Boraginaceae is an average-sized tree of tropical and subtropical origin. It is widely found in Sri Lanka, India, and other tropical regions of the world. The use of this plant has been on ancient practice for the management of a variety of human disorders. It is also an important plant species found in various traditional Indian systems of medicine including Ayurveda, Unani and Siddha. Seeds of *Cordia dichotoma Forst* are used for the management of various inflammatory disorders[4]. Fruits are used as expectorant, astringent, laxative and anthelmintic. Some common ethnomedinal uses of *Cordia dichotoma Forst* includes antidiabetic, immunomodulator, diuretic, anthelmintic, wound healing, antiulcer, gastro protective, anti-inflammatory, antidiabetic, and hepato protective and antioxidant activities. The bark of *Cordia dichotoma Forst* has been reported to a variety of phytoconstituents such as betulin, á-amyrin, octacosanol, â-sitosterol lupeol-3-rhamnoside, â-sitosterol-3-glucoside, hentricontanol, taxifolin-3,5-dirhmnoside, and hesperitin-7-rhamnoside.

Experimental work

Collection of plants

The leaves of *Cordia Dichotoma Forst*. have been collected from the local area of Toranmal Tal. Shahada. Dist.Nandurbar (Maharashtra). Crude drugs washed properly through water and dried in shade[5].

Authentification of plants

Cordia Dichotoma Forst leaves were collected freshly from Nandurbar District from Toranmal hill station of Satpuda during rainy season Maharashtra, in the month of June to October. The *Cordia dichotoma* Forst leaves were shade-dried at room temperature for seven days and the dried leaves were powdered to 22 mesh size and stored in an air tight container[6]. The plant is authentify by Dr. Santosh Tayade, Dept. of Botany, Art's, Science and Commerce College, Lonkheda, Tal.Shahada, Dist- Nandurbar (MS).

The voucher specimen for dried leaves of *Cordia Dichotoma Forst* (PSGVPM ASC-2345/02/2018) were deposited in the herbarium of Department of Botany, has been preserved in the laboratory for future reference[7, 8].

Drugs and chemicals

Folin-Ciocalteu phenol reagent (FCR, Merck), dimethyl sulphoxide (DMSO, Merck, India), phosphate-buffered saline (PBS, HiMedia), Eagle modified essential medium (EMEM media, HiMedia), fetal calf serum (HiMedia), methyl-thiazolyl-tetrazo-lium dye (MTT, HiMedia), 2,7-dichlorodihydro-fluorescein diacetate (DCFH-DA, Sigma-Aldrich,USA), 4',6-diamidino-2-phenylindole (DAPI, Hi media), Para-formaldehyde (HiMedia), TritonX-100 (Merck,India), 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazol-carbocyanine iodide (JC-1, Sigma-Aldrich, USA), tamoxifen (Tam, Sigma-Aldrich, USA), Ascorbic acid, Nitric acid, Potassium citrate, Hydrogen peroxide, Nitric oxide, Sodium nitroprusside from SDFCL, SD Fine Chemical Limited, Worli Road Mumbai. Ampiciline, Chloramphenicol, Ciprofloxacin, Norfloaxacin from USV Limited, Govandi, East Mumbai.

Instrumentation[9]

UV-spectrophotometer, incubator, microplate reader (BIORAD-680), CO2 incubator (Excella ECO-170, New Brunswick), Inverted fluorescent microscope (Nikon Eclipse Ti-S, Japan), Inverted phase contrast microscope (Nikon Eclipse Ti-S, Japan). The IR spectra were carried out by SHIMADZU PERKIN EKMER 8201 PC IR SPECTROMETER using a thin film on potassium bromide pellets. The NMR spectra were recorded on BRUKER AVANCE II 300 NMR SPECTROMETER in a mixture of CDCl3. Chemical shift values are reported as values in ppm relative to TMS (d=0) as internal standard. The FAB mass spectra were recorded on JEOL SX-102/DA-6000 Mass Spectrometer using Argon/Xenon (6Kv, 10Ma) as the FAB gas.

Isolation of active constituent from (MECD) Methanolic extracts of *Cordia Dichotoma Forst leaves* by Column Chromatography

Methanolic extract was subjected to column chromatography based on phytochemical screening and TLC pattern. The constituents of methanolic extract were isolated by column chromatography and identification and purity determination were done by TLC techniques, Infra-Red Spectroscopy, Liquid Chromatography, Mass spectroscopy and Nuclear Magnetic Resonance.

Optimization of TLC solvent system

Different solvents systems were tried for developing a TLC system for identification of constituents in the extract based on the literature survey and keeping in mind the chemical nature of the constituents and the one showing maximum separation was selected as mobile phase for the study[10].

Thin layer chromatography

The elute compound is identified with freshly prepared TLC plates by following ratio: the 100% petroleum ether followed by petroleum ether: benzene, 100% benzene, benzene: chloroform, 100% chloroform, chloroform: methanol, 100% methanol within their polarity. Prepare the TLC plates using silica gel or use the percolated TLC plates and saturate the chamber or beaker with selected mobile phase for 30 minutes. TLC results are qualitative and cannot be quantified; therefore it gives positive or negative results. The major drawback of TLC is its low sensitivity and low specificity, thus negative results of TLC are not always negative by other methods. TLC relies on a reproducible migration pattern by drug of a thin layer adsorbent (eg: silica gel coated glass plates). Characterization of a particular drug is achieved by color reaction produced by spraying the plate with coloring reagents. To accomplish this, the patient's sample is spotted along with known drugs on a TLC plate, which is put in a solvent chamber. The solvent runs up the plate, it is dried and sprayed with various reagents. The color spots of various known drugs are then compared with sample from unknown sample. The spot location is located by an Rf (retention factor) number which is the ratio of distance travelled by the solvent from the original, where the sample was spotted[11] .

Retention factor (Rf) = Distance travelled by the drug / Distance travelled by the solvent

The Rf provides corroborative evidence as to identify the compound.

TLC plates were made and elute of different fraction was checked out by difference ratio of solvent system & send the isolated compound for NMR, IR and Mass spectra. The column separation carried with silica gel C and elevation was carried out starting form pet. Ether to various compounds for non-polar to polar solvent the compound obtain in higher quantity was taken for phytochemical identification using various spectrophotometric techniques.

The Phytochemical test indicated the presence of flavones.

Analysis of Compound

Physical state : Reddish Brown solid
crystal Rf value : 0.9
Solvent system: Chloroform: Methanol (70:30)
Melting point : 125ºC
Taste : Bitter

It gives positive test for Flavonoids.

The following solvents were used for the development of the TLC system

- Methanol: Ethyl acetate (9:1)

- Butanol: G.Acetic acid: water (4:1:5)
- Toluene: Ethyl acetate: G.Acetic acid (1.4: 0.4: 0.2)
- Ethyl acetate: Formic acid: G. Acetic acid: water (1.4:0.2:0.1: 0.3)
- Methanol: water (1.8: 0.2)
- Chloroform: Methanol (1.5:0.5)
- Methanol: Ammonia (1.9:0.1)

Procedure

The isolated compound were dissolved in suitable solvent and then spotted on the pre- coated silica gel G 254 plates with the help of capillary tubes. TLC plates were developed and spots were identified in iodine chamber.

COLUMN CHROMATOGRAPHY

Principle

In column chromatography, the stationary phase is held in a narrow tube i.e. column and the mobile phase are forced through the tube under pressure or by gravity. Generally, silica gel is used as stationary phase, which is polar in nature. Substances with higher polarity adsorb more strongly to polar support of silica gel and are eluted more slowly than less polar substances. Thus the least polar substance usually elutes i.e. comes out first from the column and highly polar substance usually elutes last from the column[12]. Solvent can be removed from the eluent by evaporation to isolate the substance in relatively pure form.

Isolation of phytoconstituents from (MECD) Methanolic extract of *Cordia Dichotoma Forst.* leaves.

Preparation of column

Column chromatography in chemistry is a method used to purify individual chemical compounds from mixtures of compounds. It is often used for preparative applications on scales from micrograms up to kilograms. The classical preparative chromatography column is a glass tube with a diameter from 5 mm to 50 mm and a height of 50 cm to 1 m with a tap at the bottom. Two methods are generally used to prepare a column; the dry method, and the wet method. For the dry method, the column is first filled with dry stationary phase powder, followed by the addition of mobile phase, which is flushed through the column until it is completely wet, and from this point is never allowed to run dry. A small amount of sand was kept at the top of the column. Excess solvent was run off until the level of mobile phase fell to one cm just above the top of the sand layer. For wet the method, a slurry is prepared of the eluent with the stationary phase powder and then carefully poured into the column. Care must be taken to avoid air bubbles. A solution of the organic material is pipette on top of the stationary phase[13]. This layer is usually topped with a small layer of sand or with cotton or glass wool to protect the shape of the organic layer from the velocity of newly added eluent. Eluent is slowly passed through the column to advance the organic material. Often a spherical eluent reservoir or an eluent-filled and stopper separating funnel is put on top of the column.

Preparation of Sample loading

The methanolic extract is subjected to column chromatography packed with the silica gel (60-120 mesh)

as adsorbent in the column, dried and applied on the column to separate possible phytoconstitutents.

Procedure for the isolation

The combinations of solvent systems developed for TLC was used as mobile phase for column chromatography and column was eluted by gradient elusion methods. Column was first eluted with pure pet.ether and then gradually with increasing quantity.

The column will be eluted with the 100% petroleum ether followed by petroleum ether: benzene, 100% benzene, benzene: chloroform, 100% chloroform, chloroform: methanol, 100% methanol within their polarity. (95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40, 55:45, 50:50, 45:55, 40:60, 35:65, 30:70, 25:75, 20:80, 15:85,10:90, 5:95). The column was eluted till 95% of fraction loaded eluted out.

All the collected fractions were monitored simultaneously on TLC plate. The fractions showing same TLC Pattern were pooled to there and finally make fraction (F1-F10) were obtained. Percentage yield of collected elutes were determined with respect to total weight of fraction. Total 155 fraction were collected of 20 ml elutes. Fraction (F1-F10) subjected to preliminary phytochemical analysis & Pharmacological screening of isolated compound for Anticancer, Antioxidant, and Antimicrobial activity of Leaves *Cordia Dichotoma Forst*.

Fraction eluted by Chloroform: Methanol (70:30), showed a single spots on TLC and designated as compounds CDM-B. The spectral structure of isolated compound was identified by spectroscopy technique, IR, NMR, MASS.

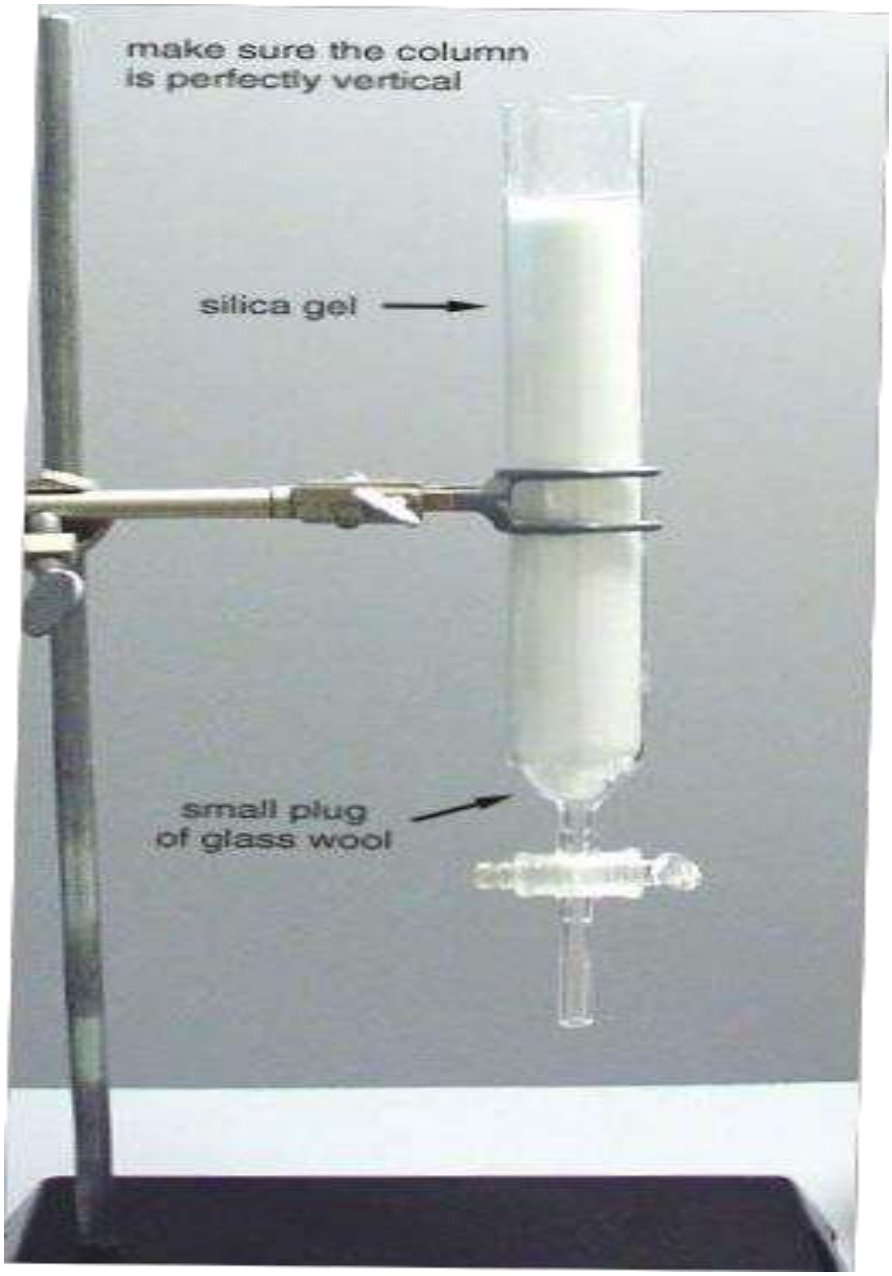


Figure No. 1: Packed column chromatography

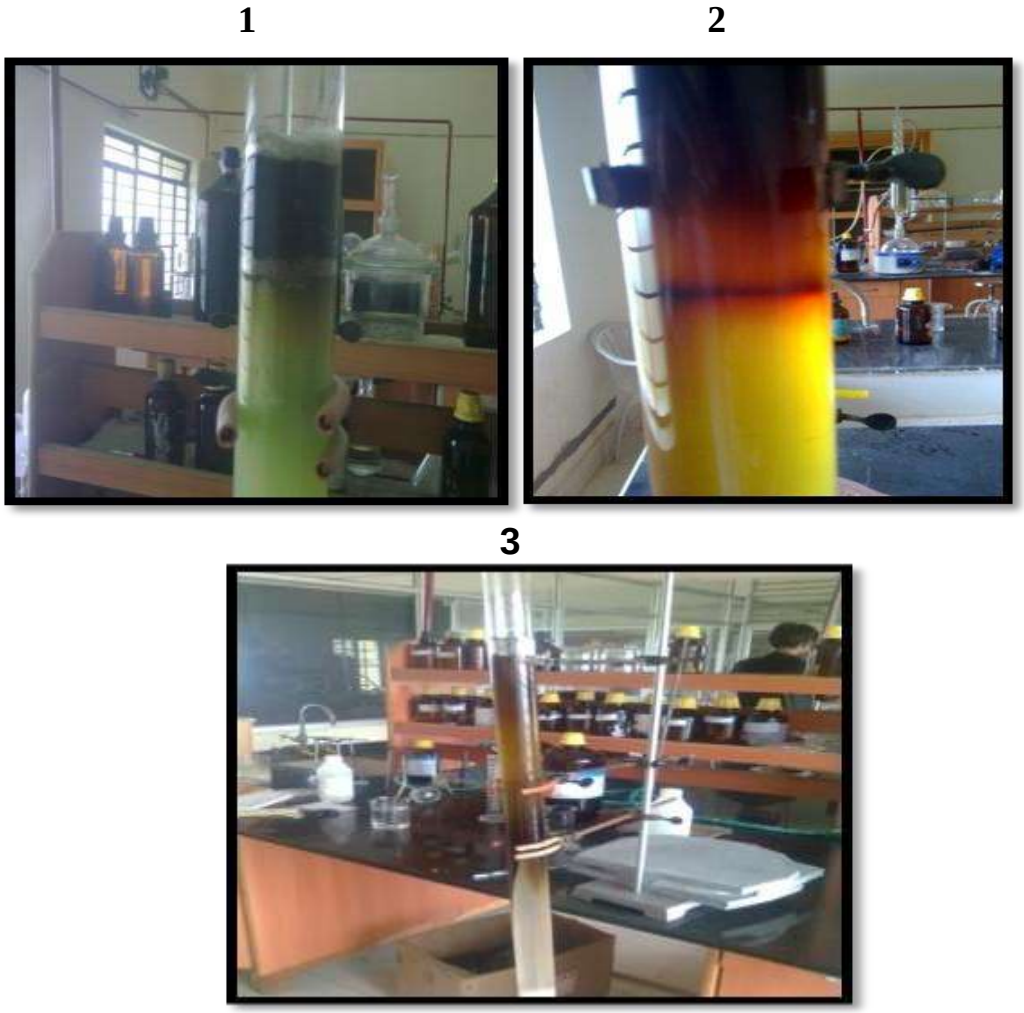


Figure No. 2: Isolation with different solvents
(1. Pet. Ether: benzene, 2. Benzene: chloroform, 3. Chloroform: methanol)

Table No.1: Column details

Sr. No	**Description**	**Details**
1	Column length	40 cm
2	Packing material	Silica gel 60-100 mesh
3	Weight of sample packing	15 gm
4	Solvent use for column packing	Pet. Ether (40 - 60 ^{O}C)
5	Volume of each fraction collection	100ml

Table No. 2: Column chromatography of methanolic extract (MECD)

Sl. No	Mobile phase	Ratio	Fraction	Nature and Weight
1.	**Pet. Ether**	**100**	**1-50**	**Yellowish brown colour (15 mg)**
2.	Pet. Ether: benzene	90:10	6-10	yellow brown colour (30mg)
3.	Pet. Ether: benzene	80:20	11-15	yellow brown colour (32mg)
4.	Pet. Ether: benzene	70:30	16-20	yellow colour (25mg)
5.	Pet. Ether: benzene	60:40	21-25	Dark yellow colour (22mg)
6.	Pet. Ether: benzene	50:50	26-30	Dark yellow colour (10mg)
7.	Pet. Ether: benzene	40:60	31-35	Dark brown colour (22mg)
8.	Pet. Ether: benzene	30:70	36-40	Dark brown colour (22mg)
9.	Pet. Ether: benzene	20:80	41-45	Dark brown colour (49mg)
10.	Pet. Ether: benzene	10:90	46-50	Light brown colour (25mg)
11.	**Benzene**	**100**	**51-55**	**Light brown colour (30mg)**
12.	Benzene: chloroform	90:10	56-60	Light brown colour (50mg)
13.	Benzene: chloroform	80:20	61-65	Light brown colour (40mg)
14.	Benzene: chloroform	70:30	66-70	Light yellow colour (15mg)
15.	Benzene: chloroform	60:40	71-75	yellow colour (15mg)
16.	Benzene: chloroform	50:50	76-80	Light yellow colour (33mg)
17.	Benzene: chloroform	40:60	81-85	Light yellow colour (44mg)
18.	Benzene: chloroform	30:70	86-90	Light yellow colour (22mg)
19.	Benzene: chloroform	20:80	91-95	Light yellow colour (20mg)
20.	Benzene: chloroform	10:90	96-100	Light yellow green colour
21.	**Chloroform**	**100**	**101-105**	**Light reddish green(15mg)**
22.	Chloroform:	90:10	106-110	Light reddish (20mg)
23.	Chloroform: Methanol	80:20	111-115	reddish brown powder (145mg)
24.	Chloroform: Methanol	70:30	116-120	reddish brown powder (485mg)
25.	Chloroform: Methanol	60:40	121-125	blackish brown mass(325mg)
26.	Chloroform: Methanol	50:50	126-130	Dark brown powder(100mg)
27.	Chloroform: Methanol	40:60	131-135	Dark reddish brown powder
28.	Chloroform: Methanol	30:70	136-140	Dark reddish brown powder
29.	Chloroform: Methanol	20:80	141-145	Reddish brown powder (125mg)
30.	Chloroform: Methanol	10:90	146-150	Reddish brown powder (110mg)
31.	**Methanol**	**100**	**151-155**	**Reddish brown powder (95mg)**
				Total = 2.352gm

Results

Optimization of TLC solvent system

Different solvents systems were tried for developing a TLC system for identification of constituents in the extract based on the literature survey and keeping in mind the chemical nature of the constituents and the one showing maximum separation was selected as mobile phase for the study[14].

Thin Layer Chromatography

The elute compound is identified with freshly prepared TLC plates by following ratio: the 100% petroleum ether followed by petroleum ether: benzene, 100% benzene, benzene: chloroform, 100% chloroform, chloroform: methanol, 100% methanol within their polarity. Prepare the TLC plates using silica gel or use the percolated TLC plates and saturate the chamber or beaker with selected mobile phase for 30 minutes. TLC results are qualitative and cannot be quantified; therefore it gives positive or negative results. The major drawback of TLC is its low sensitivity and low specificity, thus negative results of TLC are not always negative by other methods. TLC relies on a reproducible migration pattern by drug of a thin layer adsorbent (eg: silica gel coated glass plates). Characterization of a particular drug is achieved by color reaction produced by spraying the plate with coloring reagents. To accomplish this, the patient's sample is spotted along with known drugs on a TLC plate, which is put in a solvent chamber. The solvent runs up the plate, it is dried and sprayed with various reagents. The colour spots of various known drugs are then compared with sample from unknown sample. The spot location is located by an Rf (retention factor) number which is the ratio of distance travelled by the solvent from the original, where the sample was spotted.

Retention factor (Rf) = Distance travelled by the drug / Distance travelled by the solvent

The Rf provides corroborative evidence as to identify the compound.

TLC plates were made and elute of different fraction was checked out by difference ratio of solvent system & send the isolated compound for NMR, IR and Mass spectra. The column separation carried with silica gel C and elevation was carried out starting form Pet. ether to various compounds for non-polar to polar solvent the compound obtain in higher quantity was taken for phytochemical identification using various spectrophotometric techniques[15]. The Phytochemical test indicated the presence of flavones.

TLC of Methanolic extract of *Cordia dichotma* Forst.

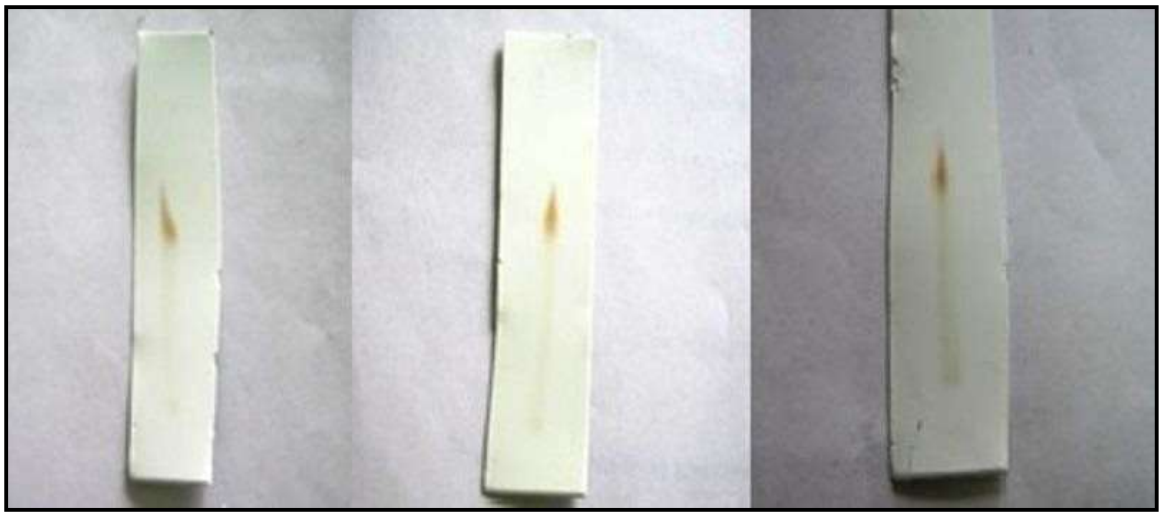
TLC study has shown the presence of different components present in methanolic extract of *Cordia dichotma Forst.* When the extract was run in specific solvent system. Before reaching to most optimum solvent system a number of systems were employed.

Table No.3 : Summary of TLC

S.No.	Solvent system	Detecting reagents	Color	No.of Spots	Rf value	Melting Point
1.	Chloroform:Methanol (70:30)	Anisaldehyde Sulphuric acid	Reddish Brown	1	0.9	125^0C

Table No.4: Spot details

Physical state	Reddish Brown solid crystal
Rf value	0.9
Solvent system	Chloroform: Methanol (70:30)
Melting point	125ºC
Taste	Bitter
It gives positive test for Flavonoids	



CH:ME 20:80 **CH:ME 70:30** **CH:ME 50:50**

Figure No. 3: TLC of Methaolic extract of *Cordia dichotoma* Forst.

Conclusion

In this study, we successfully isolated and characterized a flavonoids compound, 7-hydroxy-2-(4-nitrophenyl)-4H-chromen-4-one, from the methanolic extracts of *Cordia dichotoma Forst* leaves using column chromatography techniques. The isolated compound exhibited significant anticancer antioxidant, antimicrobial activities, making it a promising natural product for the development of new therapeutic agents. The results of this study provide valuable insights into the chemical composition of *Cordia dichotoma Forst* leaves and highlight its potential as a natural source of antioxidant and anti-inflammatory agents. The isolation and characterization of 7-hydroxy-2-(4-nitrophenyl)-4H-chromen-4-one provide evidence for the traditional use of *Cordia dichotoma Forst* leaves in folk remedies and demonstrate the potential of this plant species for the development of new therapeutic agents.

The anticancer antioxidant, antimicrobial activities of the isolated compound suggest that it may be useful in the prevention and treatment of various diseases, including inflammation, oxidative stress, and cardiovascular disease. Further studies are needed to fully evaluate the safety and efficacy of 7-hydroxy-2-(4-nitrophenyl)-4H-chromen-4-one as a therapeutic agent. The results of this study also demonstrate the potential of column chromatography techniques for the isolation and purification of bioactive compounds from plant extracts. The use of column chromatography allows for the rapid and efficient separation of complex mixtures, enabling the isolation of pure compounds with specific biological activities. In conclusion, the isolation and characterization of flavonoids 7-hydroxy-2-(4-nitrophenyl)-4H-chromen-4-one from *Cordia Dichotoma Forst* leaves provides evidence for the traditional use of this plant species in folk remedies and highlights its potential as a natural source of anticancer, antioxidant, antimicrobial agents. Further studies are needed to fully evaluate the potential of this compound as a therapeutic agent[16].

References

1. Awad AB, Chinnman M, Fink CS, Bradford PG. Beta-sitosterol activates Fas signaling in human breast cancer cells. Phytomed, 2007; 14:747–754.
2. Baskar AA, Ignacimuthu S, Paulraj G, Numair K. Chemopreventive potential of β-Sitosterol in experimental colon cancer model - an *in vitro* and *in vivo* study. BMC Comp Alt Med, 2010; 10:24.
3. Bhattacharya P, Saha A. Evaluation of reversible contraceptive potential of *Cordia dichotoma* leaves extract. Rev Bras Farm, 2013; 23(2):342-350.
4. Dayal R, Sharma M, Sharma R, Chand S. Analysis of fatty oils of the seeds of *Cordia dichotoma* and *Crataeva nurvala*. J. Lipid Sci. Tech., 2006; 38(4):183-184.
5. Dighe PA, Dighe S. A review on medicinal fruit bhokar of species *Cordia dichotoma* Forst. Int J Pharm Biol Arch, 2014;5(3): 41-47.
6. Edwards BJ. Treatment of chronic leg ulcers with ointment containing soluble chlorophyll. Physiother, 1954; 40:177–179.
7. Egner PA, Munoz A, Kensler TW. Chemoprevention with chlorophyllin in individuals exposed to dietary aflatoxin. Mutat Res., 2003; 52(3):209–216.
8. Egner PA, Wang JB, Zhu YR, Zhang BC, Wu Y, Zhang QN. Chlorophyllin intervention reduces aflatoxin-DNA adducts in individuals at high risk for liver cancer. Proc Natl Acad Sci, 2001; 98(25):1401-1406.
9. Ferruzzi MG, Blakeslee J. Digestion, absorption, and cancer preventative activity of dietary chlorophyll derivatives. Nutr Res, 2007; 27:1–12.
10. Ganjare AB, Nirmal S, Patil AN. Use of apigenin from *Cordia dichotoma* in the treatment of colitis. Fitoterapia, 2011; 82(7):1052-1056.
11. Henderson BW, Bellnier DA, Greco WR, Sharma A, Pandry RK, Vaughan LA. An *in vivo* quantitative structure-activity relationship for a congeneric series of pyropheophorbide derivatives as photosensitizers for photodynamic therapy. Cancer Res, 1997; 57:4000- 4007.
12. Jamkhande PG, Barde SR, Patwekar SL, Tidke PS. Plant profile, phytochemistry and pharmacology of *Cordia dichotoma* (Indian cherry): A review. Asian Pac J Trop Biomed, 2013; 3(12):1009-1012.
13. Jayaprakasha GK, Mandadi KK, Poulose SM, Jadegoud Y, Gowda GA, Patil BS. Inhibition of colon cancer growth and antioxidant activity of bioactive compounds from *Poncirus trifoliate* (L.) Raf. Bioorg Med Chem, 2007; 15:4923-4932.
14. Jesch ED, Seo JM, Carr TP, Lee JY. Sitosterol reduces messenger RNA and protein expression levels of Niemann-Pick C1-like 1 in FHs 74 Int cells. Nutr Res, 2009; 29(12):859-66.
15. Katolkar PP, Wanjari BE, Nimbekar TP, Duragkar NJ. Antiimplantation activity of the methanolic extract of *Cordia dichotoma* Lam. bark in rats. Int J of Biomed Adv Res, 2012; 3(3):202-204.
16. Kephart JC. Chlorophyll derivatives- their chemistry, commercial preparation and uses. Econ Bot, 1955; 9:3-18.