International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2020; 8(1): 410-413

Received: 10-11-2019
Accepted: 12-12-2019

Khan RMR
Student Vasantrao Naik College of Agricultural Biotechnology, Yavatmal, Maharashtra, India

Shinde RM
Assistant professor Vasantrao Naik College of Agricultural Biotechnology, Yavatmal, Maharashtra, India

Wasule DL
Assistant professor Vasantrao Naik College of Agricultural Biotechnology, Yavatmal, Maharashtra, India

Anjali M Gaharwar
Assistant professor Vasantrao Naik College of Agricultural Biotechnology, Yavatmal, Maharashtra, India

Parlavar ND
Associate Dean Vasantrao Naik College of Agricultural Biotechnology, Yavatmal, Maharashtra, India

Patle KP
Student Vasantrao Naik College of Agricultural Biotechnology, Yavatmal, Maharashtra, India

Corresponding Author:
Shinde RM
Assistant professor Vasantrao Naik College of Agricultural Biotechnology, Yavatmal, Maharashtra, India

Extraction and characterization of phytochemicals from *Cochlospermum* Spp. and its antifungal activity

Khan RMR, Shinde RM, Wasule DL, Anjali M Gaharwar, Parlavar ND and Patle KP

DOI: https://doi.org/10.22271/chemi.2020.v8.i1f.8281

Abstract
The work is initiate to assess the phytochemical and antifungal activity of *Chochlospermum* Spp. *Cochlospermum* Spp. is an Indian subcontinent plant used in Ayurvedic treatment for many diseases. The analysis of the secondary metabolites produced in *Cochlospermum Spp* was done using Thin layer chromatography (TLC) by standard chemical protocol. Using thin layer chromatography (TLC) different bands of secondary metabolites are observed and developed in the different solvents. Standardization of Extracts with four solvents i.e. Methanol, Acetone, Chloroform, and Petroleum ether was carried out. The antifungal activity tested against two fungus i.e. *Microfamina* fungi and *Aspergillus fumigates*. The extract showed growth inbibion against *Microfamina* fungi as compared with *Aspergillus fumigates.* This results may be helpful for rationale, use of this plant in the the modern system of health care.

Keywords: *Cochlospermum* Spp., secondary metabolites, quantitative analysis

Introduction
Medicinal plants contain different bioactive compounds which have great importance to the health of individuals and communities. Plants parts were used in villeges for curing many diseseases on the traditional basis. *Cochlospermum* Spp. Known as Buttercup, Yellow silk, Cotton tree and Golden silk. *Cochlospermum* Spp. is a genus in Bixaceae family. *Cochlospermum* Spp. Is native to tropical regions of the world, particularly Latin, America, Africa, the Indian subcontinent, and Australia. The use of this plant in traditional is very useful for many diseases. The dried bark of *Cochlospermum* Spp. a Mexican medicinal plant used to treat Jaundice, Liver ailments and hepatitis c. The presence of these compounds in plant correlets with the use of this Mexican medicinal plant [1]. *Cochlospermum* Spp. has many properties against diabetes and Hypercholesterolemia have been empirically known for many years [2]. The plant is used in the folk medicine for the treatment of hypertension, diabetes, hepatitis and releted diseases, the plant could be used as a potential agent against Metabolic Syndrome (MS) since it shows hypoglycaemic, vasorelaxant and hepatoprotective properties [3]. These-plant induces vasorelaxant and antihypertensive effects mainly by activation of NO /cGMP signalling pathway [4]. This plant is also use in the tracheal relaxation effect [5]. Flavonoids, sterols and lingams, presence of these compounds in plant correlets with the use of this Mexican medicinal plant with it 'sliver activity [6].
In this study attempt was made to extract biologically active plant secondary metabolites or phytochemical for checking its antifungal activity.

Materials and Methods
Sample Collection
The plant sample was collected from Pusad district Yavatmal in the month of April. The plant sample were thoroughly washed, air dried and then store it in air tight bottles.

Preparation of media
For isolation of fungus and maintenance of fungal culture Potato Dextrose Agar (PDA) were used.1000 ml of water boiled into a heating mantle and then add 200 g Potatoes extract into it.

Add 20 g Dextrose and lastly add 20 g of Agar and made the volume of on 1000 lit. The medium was distributed into the flasks and test tubes and sterilized in autoclave at 121 ºC for 15 psi for 15 min. The slants were used for maintenance of culture and the medium in flask for isolation of fungus on Potato Dextrose Agar was used for isolation and maintenance of pure culture of fungal pathogens.

Soxhlet Extraction

40 gm of plant sample was placed in small size of muslin cloth in Soxhlet apparatus. The extraction chamber was filled with 250 ml of an appropriate solvent such as methanol, petroleum ether, acetone and chloroform through the open end so that the plant sample was properly deeped in it. The extraction was carried out until the extract was colourless. The extracts were placed in water bath (at boiling point of solvent for 3 hours) by using bowls to evaporate the solvent and stored at 4° C for further analysis.

Thin Layer Chromatography (TLC)

Thin layer chromatography was carried out to know the chemical profile of methanol chloroform, petroleum ether and acetone extract of *Cochlospermum* Spp. And also to know the chemical compounds present in it

Preparation of TLC plates:

The TLC plates were prepared. Briefly, 25 g of silica gel-G was mixed with 50 ml of distilled water and the slurry formed was uniformly spread over by using the spreader on TLC plates of 0.25 mm. The plates were allowed to dry at room temperature and heated in an oven at 100 °C for 2 hrs [8].

Standardization of solvent system

The crude extract of (*Cochlospermum Spp*) plant sample were diluted in respective solvent. The prepared TLC plates were marked 1 cm from bottom and 10 ul each sample was applied on TLC plates at equal distance with the help of capillary tubes. For separation of maximum bands on TLC plates different solvent systems were used according to polarity and from that Chloroform: Ethyl acetate: Acetic acid (50:50:1), Toluene: Ethyl acetate (93:7), for (*Cochlospermum Spp)*. Extract were selected as standard solvent system. TLC plates were kept in chromatography chambers, containing Chloroform: Ethyl acetate: Acetic acid (50:50:1), Toluene: Ethyl acetate (93:7) as solvent system for respective extract and allowed to run until it reached as 3/4th position. The developed chromatogram on TLC plates was allowed to air dry and observed under visible, UV light. The bands were noted.

Agar well diffusion assay for checking Effects of Plant Extract on *Microfamina fungi* and *Aspergillums fumigatus*

In Agar well diffusion assay the efficacy of partially purified extract of Cochlospermum Spp. at 10mg and 20mgconcentrations was studied in Methanol and Acetone solvent. All concentrations 10 and 20 mg/ml extract were tested against test fungus under *in vitro* condition following on agar well diffusion method. 400 ml of Potato dextrose agar was prepared in 500 ml of 2 conical flasks and sterilized in autoclave at 121 °C at 15 psi for 15 min. After that the media poured in petri plates 20ml in each plate for each conc. three replicates were use. After solidification of media five wells (one in middle and four on side) were aseptically punctured by using sterile borer and different concentrations (10,20 µl) of extracts were loaded into the middle one Well and fugal culture were inoculated in four Wells of side. For each concentration, three replicates (plates) were used. The plates were incubated at 37 °C for 24 hrs and the zone of inhibition was measured around the wells [7].

Results and Discussion

Pure culture *Microfamina fungi* and *Aspergillums fumigatus*

The pure culture of test fungus were maintained in PDA plates. The slants were prepared for repeated subculturing.

Extraction yield

The Methanol and petroleum ether was found extensively useful for extraction yield and was the most capable to extract more substances that preferably dissolved in Methanol and in petroleum ether. Extraction was done using soxhlet apparatus method.

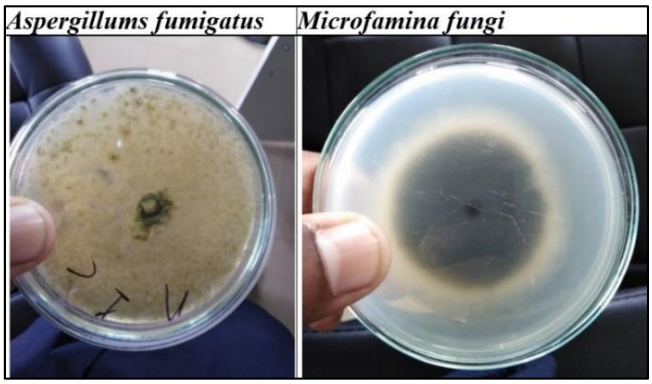

Fig 1: Pure cultures of *Aspergillums fumigates and Microfamina fungi*

Thin Layer Chromatography (TLC)

Thin layer chromatography was used for separation of different chemical constituents present in Methanol, petroleum ether, chloroform and acetone extract of *Cochlospermum* Spp.

Standardization of solvent system

Different solvent systems were screened for the efficient separation of bands according to polarity. Total four (4) solvent systems were used in present investigations to know the most suitable solvent system for separation of compounds in methanol, petroleum ether, and acetone and chloroform extract of *Cochlospermum* Spp. The results are in line with the Pooja Ponnammma *et al*. 2017 [9].

Table 1: Standardization of solvent system for methanolic extract of *Cochlospermum* Spp.

Sr. No.	Solvent system	Proportion	Methanolic extract	
			Rf values	Colour
1.	Chloroform: Ethyl acetate: Acetic acid	50: 50: 1	0.72	Pale Yellow
2.	Toluene: Ethyl acetate	93: 7	0.69	Dark Yellow
3.	Ethyl acetate: Acetone	4: 6	0.65	Whitish Yellow
4.	Toluene: Ethyl acetate: Formic acid	7: 2. 7: 0.3	0.65	Gold Yellow

Table 2: Preliminary Phytochemical screening of *Cochlospermum* Spp.

Test	Phytochemical test	Methanol	Chloroform
Tannins	$FeCl_3$ Test	-	+
Flavonoids	Shinado Test	+	-
Phenols	FC reagent test	+	+
Saponins	Foam Test	+	-
Alkaloids	Mayers Test	-	+

The phytochemical screening was carried out using $FeCl_3$ Test, Shinado Test, FC reagent test, Foam Test and Mayers Test; which confirms the results of pooja *et al*. 2017.

Agar well diffusion assay for checking Effects of Plant Extract on *Microfamina fungi and Aspergillus fumigatus*

Aspergillus fumigates which showed Growth inhibition of only 4.5%, In Comparison *Microfamina* fungi was found to be more which was 9.8% in methanol extract at the extract concentration of 10 mg/ml as compared with *Aspergillus fumigatus*. Whereas at the concentration of 20 mg/ml maximum growth inhibition was found against *Microfamina.*

Table 3: Growth inhibition % of *Cochlospermum* Spp.

Growth inhibition %					
Extract Concentration mg/ml	Control	Microfamina		*Aspergillus fumigatus*	
		Methanol extract	Acetone extract	Methanol extract	Acetone extract
10	00	9.8	3.3	4.5	2.2
20	00	16.2	7.7	8.5	5.7

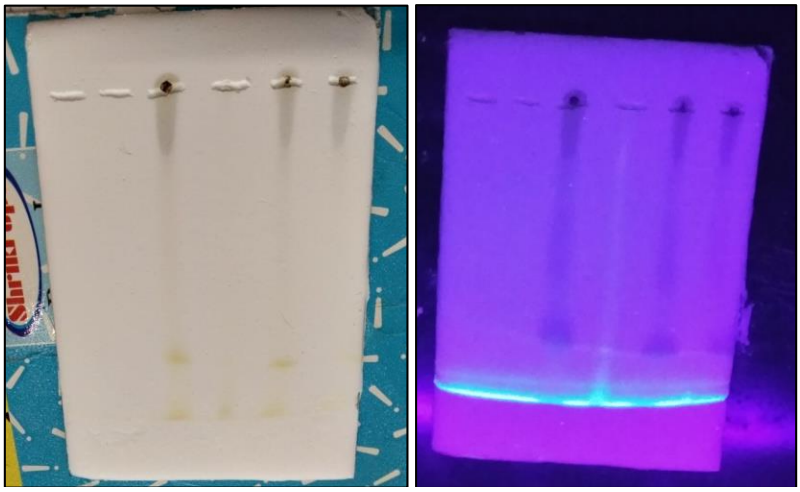

Fig 2: TLC Plate before kepting in Chamber [Chloroform: Ethyl acetate: Acetic acid (50:50:1)]

Conclusion

The Methanol as a solvent was found to be most effectively for high extract yield of *Cochlospermum* Spp. With their antifungal activity. The solvent systems Chloroform: Ethyl acetate: Acetic acid (50:50:1) were effective for good separation of bands of secondary metabolites by thin layer chromatography. Methanolic extract showed antifungal activity against *Microfamina* in an Agar well diffusion method. *Cochlospermum* Spp. Methanolic extract Have a good antifungal activity than that of the chloroform, acetone and petroleum ether extract.

Acknowledgements

We wish to thank Dr. N.D. Parlavar, Associate Dean, Vasantrao Naik College of Agricultural Biotechnology, Yavatmal, Maharashtra, India for financial support, valuable encouragement and kind assistance.

Reference

1. Deharo E1, Baelmans R, Gimenez A, Quenevo C, Bourdy G. *In vitro* immunomodulatory activity of plants used by the Tacana ethnic group in Bolivia.
2. Baños G1, Pérez-Torres I, El Hafidi M. Medicinal agents in the metabolic syndrome.
3. Sánchez-Salgado JC1, Ortiz-Andrade RR, Aguirre-Crespo F, Vergara-Galicia J, León-Rivera I, Montes S *et al*. Hypoglycemic, vasorelaxant and hepatoprotective effects of *Cochlospermum* Spp. (wild.) Sprengel: a potential agent for the treatment of metabolic syndrome.
4. Sánchez-Salgado JC1, Castillo-España P, Ibarra-Barajas M, Villalobos-Molina R, Estrada-Soto S. *Cochlospermum* Spp. induces vasorelaxant and antihypertensive effects mainly by activation of NO/cGMP signaling pathway.
5. Sánchez-Recillas A1, Mantecón-Reyes P1, Castillo-España P2, Villalobos-Molina R3, Ibarra-Barajas M3, Estrada-Soto S4 *et al*. Tracheal relaxation of five medicinal plants used in Mexico for the treatment of several diseases.
6. Aguilar-Guadarrama AB1, Rios MY2. Flavonoids, Sterols and Lignans from *Cochlospermum* Spp. and Their Relationship with Its Liver Activity.
7. Poja Ponnamma, Manasa G, Sudarshana MS, Murali M, Mahendra C. *In Vitro* antioxidant, antibacterial and phytochemicak screening of Cochlospermum religiosum (L.) Alston – A potent medicinal plant
8. More Y, Gade RM, Shitole AV, Wahare SH. Phytochemical Investigation and Thin layer

chromatography of Aegle Methanolic Extract. Advances in life Sciences. 2016; 15(15):5685-5690.
9. Poja ponnamma, Manasa G, Sudarshana MS, Murali M, Mahendra C. *In Vitro* antioxidant, antibacterial and Phyto chemical screening of Cochlospermum religiosum (L.) Alston – A potent medicinal plant.