e-ISSN: 2455-667X

Annals of Natural Sciences
Vol. 2(2), June 2016: 30-33
Journal's URL: http://www.crsdindia.com/ans.html
Email: crsdindia@gmail.com

Annals of Natural Sciences

ORIGINAL ARTICLE

Analysis of Antibacterial Activity of *C. procera* and *C. gigentia* Species Against *S. aureus* Using Chromatogrphy Separated Extracts

Navdeep Ranjan[1], Sushil Kumar Singh[1] and Shivendra Singh[2]
[1]Department of Biotechnology, A.N. College, Patna, Bihar
[2]Department of Biotechnology, R.B.S. College, Agra, U.P.
Email: shivendra_biotech@yahoo.co.in, ibrcagra@gmail.com

ABSTRACT

The present study shows the antibacterial activity of C.procera and C.gigentia on pathogenic bacteria s. aureus. After maceration of C.procera and C.gigenteathe crude extract was obtained. Further, chromatographic technique is applied and obtained 10 different farctions . By these fractions the sensitivity discs are prepared for antibacterial activity on s.aures plate. The zone of inhibition revels that the 10th fraction have maximum zone of inhibition. This fraction is further may be isolate for the antibacterial purpose.

Key word: *C. procera, C. gigentia, S. aureus, chromatography*

Received: 5th April 2016, Revised: 21st April 2016, Accepted: 25th April 2016

How to cite this article:
Ranjan N., Singh S.K. and Singh S. (2016): Analysis of Antibacterial Activity of *C. procera* and *C. gigentia* Species Against *S. aureus* Using Chromatogrphy Separated Extracts. Annals of Natural Sciences, Vol. 2[2]: June, 2016: 30-33.

INTRODUCTION

Calotropis (Family: Asclepiadaceae) is a plant growing in the wild and has been used in the traditional medicine system for the treatment of various diseases. The secondary metabolites are biologically active and structurally unique compounds which may be useful for generation of new medicines. Secondary metabolites of *Calotropis* have well known pharmaceutical and therapeutic applications. Chemical constituents of plants such as Steroid, Terpenoids, Resin, Glycosides, Carbohydrates, Aluminum, Iron, Magnesium, and Sodium are reported in Ayurvedic literature. These chemicals are reported to be Analgesic, Resilient, Anti- inflammatory, Schizontocidal Activity (Sharma & Sharma 1999), Emetic, Expectorant, Stomachic, Digestive, Laxative and Depurative. Further, these phytoconstituents are also reported potentially active for the treatment of several diseases such as Skin disease, Jaundice (Jan, *et al.* 2009), Leucoderma, Eczema, Ulcer, Piles, Dysentery (Khan 2009), Dropsy, ring worm (Kuta 2008) and Removing Thorn from body (Rai, *et al.* 2000). *Calotrois* root bark is very largely used as a treatment for elephantiasis and leprosy. The latex is as potent as standard anti-inflammatory drug Phenylbutazone (PBZ) in inhibiting inflammatory response induced by various inflammagens in acute and chronic models of inflammation. The Fresh leaves are used in treatment of Rheumatoid, Arthritis and Healing of wounds (Patil, *et al.* 2009).

MATERIAL AND METHODS

1. Plant material:

Calotropis gigantia and calotropisprocera leaf was collected in the month of January 2013 from A.N. College campus. The plant material was identified at the field using standard keys and descriptions.

2. Preparation for extract:
The fresh, healthy and diseases free leaves from different species of Calotropis were plugged aseptically by wearing gloves. All leaves were washed in running tap water. 70 % Ethanol were used for surface sterilization of leaves. After that all leaves were kept safely for dry at room temperature. Well dried leaves were subjected to fine powder by using grinder. The fine powder is kept in air tight bottle for further use.

3. Method of extraction:
Solvent- Petroleum ether, Methanol, ethyl acetate
Method- Maceration
Procedure- Leaf powder was weighed 500 gm and kept in a container in contact with 1000 ml pet ether for seven days, with vigorous shaking at regular interval. Material was filtered a first with muslin cloth and then with filter paper. Filtrate was collected and dried in water bath till no further reduction in mass of extract was observed. Dried extract was weighed and packed in air tight container and the marc was air dried then kept in a container in contact with 500 ml methanol for seven days, with vigorous shaking at regular interval. Material was filtered a first with muslin cloth and then with filter paper. Filtrate was collected and dried in water bath till no further reduction in mass of extract was observed. Further the marc was air dried then kept in a container in contact with 250 ml ethyl acetate for seven days, with vigorous shaking at regular interval. Material was filtered a first with muslin cloth and then with filter paper. Filtrate was collected and dried in water bath till no further reduction in mass of extract was observed. Dried extract was weighed and packed in air tight container.

COLUMN CHROMATOGRAPHY

Chromatography is a powerful technique for separating mixtures. There are different types of chromatography, such as paper, thin layer, or column chromatography (amongst others), each with its own strengths and weaknesses. Chromatography systems have a stationary phase (which can be solid or liquid) and a mobile phase(usually liquid or gas).
In column chromatography, the stationary phase, a solid adsorbent, is placed in a vertical glass (usually) column and the mobile phase, a liquid, is added to the top and flows down through the column (by either gravity or external pressure). Column chromatography is generally used as a purification technique: it isolates desired compounds from a mixture.

Fig. 1: The Column Chromatography setup for both *C.procera* and *C.gigentia*

Column chromatography is separated into two categories, depending on how the solvent flows down the column:-

1. Gravity Column Chromatography:
If the solvent is allowed to flow down the column by gravity, or percolation, it is called gravity column chromatography.

2. Flash Chromatography:
If the solvent is forced down the column by positive air pressure, it is called flash chromatography.

For column chromatography the extract was loded (1gm) on silica gel column packed with hexane and eluted with hexane and chloroform (9:1 to 1:9 and 100% chloroform) followed by ethyl acetate and methanol (9:1 to 1:9 and methanol) to yield 10 fractions individual fraction was collected and tested reviled that the 10th fraction that was eluted with ethyl acetate and methanol (8:2) had maximum activity.

MUELLER HINTON AGAR (MHA)

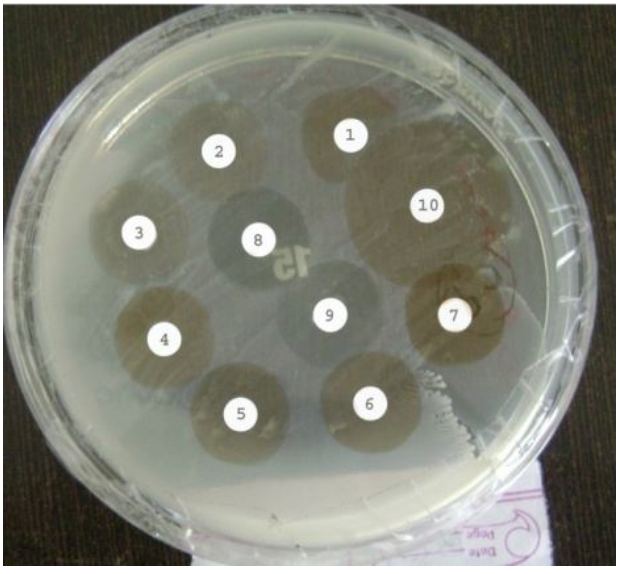
Müller-Hinton agar is a microbiological growth medium that is commonly used for antibiotic susceptibility testing.

Composition of MHA:

Ingredients	**Quantity**
Beef Extract	2.00 gm
Acid Hydrolystate of Casein	17.50 gm
Starch	1.50 gm
Agar	17.00 gm
Distilled Water	1000.00 ml

Final pH 7.3 ± 0.1 at 25ºC

Fig. 2: Antibacterial activity against *S.aureus* on Muller Hinton media



Preparation of MHA:

1. Weighed all the ingredients and added in a conical flask containing one liter of distilled water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.

3. Autoclave at 121°C for 15 minutes. Cool to room temperature.
4. Pour cooled Mueller Hinton Agar into sterile petri dishes on a level, horizontal surface to give uniform depth.
5. Allow to cool to room temperature.
6. Check for the final pH 7.3 ± 0.1 at 25ºC.
7. Store the plates at 2-8 ºC.

Quality control of MHA:

	Expected Results
Positive Control-	
Staphycoccus aureus	Good Groth, Cream Coloured Colonies
Negetive Control-	
Un-Ioculated Medium	No Change

PREPARATION OF SENSITIVITY DISCS

The sensitivity discs were prepared using sterile Whatman's No. 1 filter paper (Cheesbrough, 2002). The discs (6.0 ± 1.0 mm in diameter each) were prepared by punching the filter paper appropriately. Ten discs were dispensed into each farctions of column chromatography .
(impregnation) by means of sterile forceps.

TEST ORGANISMS

Clinical isolates of bacteria were used for the bioassay studies. The isolates Staphylococcus aureus were obtained from IBRC lab. They were further confirmed using standard biochemical tests (citrate utilization, coagulase, oxidase and catalase) as described by Cheesbrough (2002). The isolates were maintained on freshly-prepared nutrient agar (oxoid) slants and kept in a refrigerator at 4ºC until required for use.

RESULT & DISCUSSION

The crude ethyl acetate extract 50µl showed remarkable antibacterial activity with zone of inhibition a uv-visible spectrum at 200 – 300nm in a uv- visible spectrum revealed that fraction 10^{th} (Ethyl acetate : methanol = 8 : 2) as the most potent one as the zone of inhibition is above 18mm .

CONCLUSION

The present study shows that the plant extracts containchemical constituents of pharmacological significance which leads to new antibacterial drug.The presence of these chemical constituents in thisplant is an indication that the plant, if properlyscreened using additional solvents, could yield drugsof pharmaceutical significance. Further research istherefore recommended to isolate, purify andcharacterize these chemical constituents with a viewto supplementing conventional antibacterial drug development.

REFERENCES

1. Jan G., Khan M.A. and Gul F. (2009): Ethnomedicinal plants used against jaundice in Dir Kohistan.Valleys (NWFP), Pakistan, *Ethnobotanical leaflets*, 13, 1029-41.
2. Khan F.M. (2009): Ethnoveterinary medicinal usage of flora of greater Cholistan Desert Pakistan, *Pak.*
3. Kuta F.A. (2008): Antifungal effects of calotropis procera stem back on Epidermophyton, Flocosum and Trichophyton, gypseum. African. Journal of Biotchnology.7(13):2116-2118.
4. Patil S.B., Naikwade N.S., Kondawar M.S., Magdum C.S. and Awale V.B.(2009): Traditional uses of plants for wound healing in the Sangli District, Maharashtra, *I. J. Pharm. Tech. Research*. 1(3): 876-878.
5. Rai M.K., Pandey A.K. and Acharaya D. (2000): Ethnomedicinal plants used by Gond tribe of Bahanalehi District Chhinwara, M.P. J. of nontimber forest, 7(3/4): 237-241.
6. Sharma P. and Sharma J. D. (1999): Evaluation of in vitro schizontocidal activity of plant parts of calotropis procera- an ethanobotanical approach, journal of Ethnopharmacology 68: 83–95.