www.aureoleonline.in, ISSN: 2249 - 7862 (Print): 2455-877X(online)
Aureole, Vol. IX, December 2017 PP 40-45

EXTRACTION, CHARACTERIZATION AND ANTI BACTERIAL STUDIES OF CHLOROFORM EXTRACT OF ROOT OF NAREGAMIA ALATA

*** Aby Jimson**
****Preena C.V.**
*****Sreya Joseph**

Abstract

In this work we isolated and characterized the active components of the roots of *Naregamia Alata* plant and studied its antibacterial properties. The active component was isolated using chloroform in a soxhelet apparatus. The crude component was tested for components using Thin Layer Chromatography and confirmed the presence of only one component. Then the component was analyzed for antibacterial property against stains of *Escherichia Coli* and *Streptococcus*. The component was then characterized using spectroscopic techniques. The component showed antibacterial activity.

Keywords *: Naregamia Alata, Chloroform, Soxhelet, Thin Layer Chromatography (TLC), Streptococcus, Escherichia Coli, Spectrum.*

* *Assistant Professor, P.G department of chemistry. St Stephens College, Uzhavoor, Kottayam, Kerala*
** *Post Graduate student, department of chemistry. St Stephens College*
*** *Post Graduate student, department of chemistry. St Stephens College*

INTRODUCTION

Throughout the ages humans depended on nature for sustaining their life. Nature has been the source for everything that humans enjoy. Nature has been the source for a wide variety of medicines also. Medicinal plants are of ample use in health sector. Men have exploited the healing properties of medicinal plants for centuries. *Naregamia alata* (Nila-Naragam in Malayalam) is a shrub endemic to the western Ghats and that forms branches and reaches height up to 30 cm (Shinya et al, 2012; Sukumaran 2018, Geetha and Rameshkumar 2010). It is used to cure various diseases like cough, bronchitis, asthma, ulcers etc (Shinya et al, 2012; Sukumaran 2018, Geetha and Rameshkumar 2010, Visalan 2017, Nyman et al, 2001;). It is also known to be used to treat Diarrhea (Khare 2004) .

Taxonomical classification

Kingdom	**Plantae**
Division	**Tracheophyta**
Class	**Magnoliopsida**
Order	**Routine**
Family	**Meliaceae**
Genus	**Naregamia**
Species	**Alata**

The plant is widely distributed across south India up to an altitude of 900$m^{2.}$This is a small shrub growing in rainy season. Flowers are seen in May and fruits in October season (Aiyer 1994;Warrier et al. 1995)

MATERIALS AND METHODS

Plant material

Fresh whole plant was collected by April 2017 from the local fields at Uzhavoor, Kottayam district. The roots where chopped, washed with water and dried in shade for 3 weeks to remove moisture and to preserve the properties by avoiding direct sunlight. Dry roots where powdered.

Extraction of components

The root powder was the taken in a cotton bag and the components where extracted with chloroform in a soxhelet apparatus for 72 hours. The chloroform extract was then collected and solvent was dried off. The solid sample was analyzed for components.

Identification of number of components

Thin Layer Cromatography was used to identify the number of components. 8:2 mixture of Toluene: Ethyl acetate was used to analyze the extract and only one spot was obtained which indicates the presence of only one component. The component was then used for antibacterial studies.

Antibacterial Study

Bacterial strains used

1) *Streptococcus*- A gram positive bacteria belonging to phylum Firmicutes and the order Lactobacillus. These bacteria's are found in human body including nose, skin and genitals.

2) *Escherichia Coli* - A gram Negative bacteria commonly found in lower intestine of warm blooded animals and is a facultative anaerobic, rod shaped bacteria of the genus Escherichia. Most E.coli strains are harmless but some can cause serious food poisoning. The harmless strains are helpful in producing Vitamin K2.

Anti-bacterial activity of the component

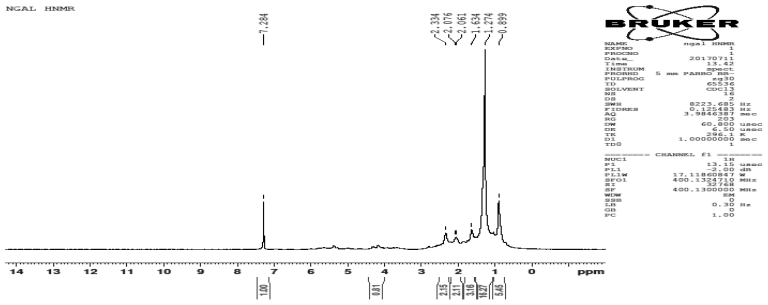
The component after evaporating the solvent was used to test its anti bacterial properties. Agar well diffusion method was used to determine the growth and inhibition of bacteria by the component. Sterile nutrient agar media was poured into a sterile petri dish. MHA plate was also prepared for bacteria, after solidification of the bacterial culture for 6 hours, were swabbed into the plated using sterile cotton. Bacteria were streaked in the MHA plates. Wells were made by using sterile well cutter. 200 µL of the component was added to the well using a sterile micro pipette. The plate was incubated at 37°C for 24 hours. The diameter of the inhibitory zone was measured in millimeters.

RESULT AND DISCUSSION

Spectral analysis of the component (Colin and Elamine 2011; Pavia et al. 2007))

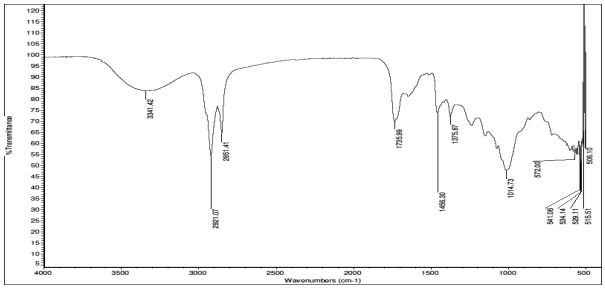
1. **^{1}H NMR spectra**
   - The NMR spectrum shows a peak at 7.284 is may be due to the presence of a highly de shielded /aromatic ring proton
   - 2 proton singlet at 2.334
   - 2 proton doublet at 2.076
   - 3 proton singlet at 1.634

• 16 proton singlet at 1.274
• 6 proton singlet at 0.899



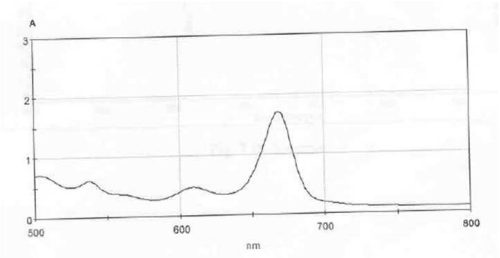
2. Infra Red spectra

• Band at 2921.07 cm-1 may be due to the presence of aliphatic C-H bonds
• Band at 3341 cm-1 may be due to the presence of N-H bond or O-H bond



3. UV- Visible spectra

The absorption maxima was observed at 668 nm



2. Anti bacterial property of the component

The component produced zone of inhibition which indicated the presence of anti microbial activity.16-17 A Zone of Inhibition of 24 mm was produced for the component against Streptococcus and zone of inhibition of 19 mm was produced against Escherichia coli. The component was shown to possess sufficient anti bacterial property and therefore send for spectroscopic analysis.

CONCLUSION

The present work Extraction, Characterization and Anti bacterial studies of Chloroform Exatract of root of *Naregamia alata* have been done. Chloroform was the solvent used for soxhlet extraction. The extract was collected and analysed by TLC and showed the presence of only one component. The characterization of this component was accompanied by spectroscopic methods like IR, UV, ^{1}H NMR Spectroscopy. The component was then subjected to anti bacterial activity and showed to be active against E.Coli and Streptococcus sp.

REFERENCE

1. Aiyer M.N , Namboothiri, A. N and kolammal. M, 1957. Pharmacology of ayurvedic drugs, Trivandrum. , 1994
2. Colin N N Banwell, Elamine M McCash,2011, "Fundamentals of molecular spectroscopy" Tata McGraw Hill education P Limited, New Delhi.
3. Geetha B. S. and K. B. Rameshkumar 2010, Essential oil composition of Naregamia alata, Phytochemistry and Phytopharmacology Division, Tropical Botanic Garden and Research Institute, Palode, Thiruvananthapuram, Kerala- 695 562, India.
4. Khare, C.P . 2004. Encyclopedia of Indian Medicinal Plants, Rational western Therapy, *Ayurveda and other traditional usage*, Springer, New Delhi
5. Nyman U, Palpu P, Joshy P, Varghese G.2001, In vitro screening of Indian Medicinal plants for antiplasmodial activity. *J Ethnopharmacol*
6. Pavia L. Donald Lampman M. Gary, Kriz S. George Vyvyan R. James 2007, "Spectroscopy" Cenage, New Delhi.
7. Shinya RV, Jomy Jose and Padmaja.V. 2012, Pharmacognostical and Biotechnological studies on Naregamia alata.. Journal of pharmaceutical and biomedical sciences *J Pharm Biomed Sci.* ; 25(25); 246-251.
8. Sukumaran S. 2008, Rare and endemic plants in the sacred groves of the Kanyakumari district in Tamilnadu. *Indian journal of forestry* , Vol 31 (4) 611-616-
9. Visalan A 2007, Traditional remedies of Kanio tribes of kottor reserve forest, Agasthyavanam, Thriruvananthapuram, Kerala. *Indian jopurnal of traditional knowledge*, 64

10. Warrier P.K ; VPK Nambiar; C Raman kutty. 1995, Indian medicinal plants: a compendium of 500 species, orient longman publishers, kottakkal, India.
11. http://microchemlab.com/test/zone-inhibition-test-antimicrobial-activity
12. https://amrls.cvm.msu.edu/microbiology/detecting-antimicrobial-resistance/test-methods/examples-of-antibiotic-sensitivity-tesing-methods