EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES

http://www.ejbps.com

ISSN 2349-8870
Volume: 7
Issue: 5
410-413
Year: 2020

FORMULATION AND ANTIMICROBIAL ACTIVITY OF *ALSTONIA SCHOLARIS* GEL

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Article Received on 04/03/2020 **Article Revised on 25/03/2020** **Article Accepted on 14/04/2020**

ABSTRACT

The present study focuses on the antimicrobial activity of different leaf extracts and formulated gels of *Alstonia scholaris* was evaluated by agar well diffusion method against pathogenic species of Gram positive bacteria viz. *Staphylococcus aureus* and Gram negative bacteria viz. *Klebsiella planticola*. The different extracts prepared were chloroform extract, acetone extract, ether extract and methanol extract and the gel of corresponding extracts were also prepared. From the study it was observed that *Alstonia scholaris* gel containing methanol extract exhibited greater antimicrobial activity as compared to that of chloroform and acetone. As well as the antimicrobial activity of the formulated gels reveals that they possess potential broad spectrum antimicrobial activity.

KEYWORDS: *Alstonia scholaris*, Antimicrobial activity, Gel, Extracts, Agar diffusion method.

INTRODUCTION

Alstonia scholaris is an evergreen, tropical tree native to the Indian subcontinent and Indomalaya, Malesia and Australia, belonging to the family Apocynaceae.[1] The *Alstoniascholars* is a glabrous tree and grows up to 40m tall, its mature bark is grayish and its young branches are copiously marked with lenticels.[2] The upperside of the leaves are glossy, while the underside is greyish. Pedicels are usually as long as or shorter than calyx. The corolla is white and tube-like, 6–10 mm; lobes are broadly ovate or broadly obviate, 2-4.5 mm, overlapping to the left. The ovaries are distinct and pubescent. The follicles are distinct and linear. Flowers bloom in the month October. The flowers are very fragrant similar to the flower of *Cestrum nocturnum* and contains alkaloids, tannins, glycosides, triterpenoids, flavonoids and phenolic acid. Bark yield the alkaloids echitenine, ditamine; crystalline and toxic echitamine; ditaine; and an uncrystallizable and bitter principle. Study isolated from the mother-liquors of echitamine hydrochloride, a crystalline alkaloid, and echitamidine.[3,4] A petroleum ether extract yielded echikautschin, echicerin, and echiretin. The bark contains indole alkaloids, including reserpine, echitamine, alstonine, tetrahydroalstonine, alstonidine, yohimbine and others. Antihypertensive effect may be due to reserpine and echitier amine. A study revealed three new indole alkaloids: nareline ethyl ether, 5-epi-nareline ethyl ether and scholarine-N (4)oxide and reported as antimicrobial, antiamoebic, antidiarrheal, antihypertensive, antimalarial, febrifuge, stimulant, hepatoprotective, immune modulatory, anti-cancer, antiasthmatic, antioxidant, analgesic, antiinflammatory, anti-fertility, anti-diabetic, cardiotonic.[5] Bitter bark and latex considered tonic and antiseptic Ditamine or ditanin considered to possess antiperiodic properties equal to the best sulphate of quinine without the latter's disagreeable side effects.The main uses include, the bark is regarded as a remedy for fevers, chronic diarrhea and dysentery. Earlier Spanish records report the dilta bark alkaloid was used in hospitals as a quinine substitute.

A gel is a solid or semisolid system of at least two constituents, consisting of a condensed mass enclosing and interpenetrated by a liquid.[6] Gel are relatively newer class of dosage form created by entrapment of large amounts of aqueous hydro alcoholic liquids in a network of colloidal solid particles, which may consists

of inorganic substances, such as aluminum salts or inorganic polymer of natural or synthetic origins like the natural gums, tragacanth, carageenin, pectin, agar and alginic acid, semi synthetic materials such as methyl cellulose, hydroxyl ethyl cellulose, hydroxyl propyl cellulose and a synthetic polymer carbapol.

A number of polymers are used to provide the structural network that is the essence of a gel system.[7,8,9] These include natural gums, cellulose derivatives, and carbomers. Although most of these function in aqueous media, several polymers that can gel nonpolar liquids are also available. Certain colloidal solids behave as gallants as a result of asymmetric flocculation of the particles. High concentration of some nonionic surfactants can be used to produce clear gel in systems containing up to about 15% mineral oil .These are employed mostly as hair dressings.

MATERIALS AND METHODS

Collection of plant materials

The fresh leaves of *A. scholaris* were collected in the month of January (2015) from the plant growing near to Nehru College of Pharmacy, Pampady, Thrissur, Kerala, India .The plant was identified with the help of Dr. Sr. Kochuthressia, Head of Botany Department, Vimala college, Thrissur and a voucher specimen has been deposited at the Botany department of the university. The Plant samples were washed and shade dried.

Preparation of extracts and phytochemical screening

The dried plant material was pulverized into fine powder using a grinder (mixer). About 50 g of powdered material was extracted in soxhlet extraction apparatus with 250 ml of each of the following solvents; chloroform, acetone and methanol.[10,11] The extracts obtained with each solvent were filtered through Whatman filter paper No. 1 and the respected solvents were evaporated (at 40ºC) with the help of heating mantle. The sticky greenish-brown substances were obtained and stored in refrigerator.[12] Some of the extracts of each solvent were used for the qualitative phytochemical screening for the identification of the various classes of active chemical constituents, using standard prescribed methods.[13] The positive tests were noted as weak (+), moderate (++), strong (+++) and absent (-).

Formulation of gel

Gel forming polymer such as carbopol 934 was soaked in water at ratio1:100 for 2 hours and then dispersed by agitation with the aid of magnetic stirrer to get a uniform dispersion. The stirring was stopped and allowed to stand for 15 min to expel the entrapped air. To this aqueous solution, triethanolamine (2% v/v) was slowly added. At this stage, prepared extract were incorporated to get the gel.[14,17]

Tested microorganisms

Various cultures of human pathogenic, gram positive and gram negative bacteria were used. These are *Staphylococcus aureus* and *Klebsiella planticola*.[15] The microorganisms were repeatedly subcultured in order to obtain pure isolates. A loop full test organism was inoculated on nutrient broth and incubated for 24 h at 37 ± 1ºC and maintained in sterile condition.[16]

Screening for antibacterial properties

Antibacterial activities of plant extracts and formulated gels were tested by agar well diffusion method.[17,18] The culture plates were prepared by pouring 20 ml of sterile nutrient agar. 1 ml inoculum suspension was spread uniformly over the agar medium using sterile glass rod to get uniform distribution of bacteria. A sterile cork borer (6 mm) was used to make wells in each plate for extracts. These plates were labeled and 100 µl of each plant extracts and formulated gel was added aseptically into the well. Then the plates were incubated for 24 h at 37ºC during which the activity was evidenced by the presence of zone of inhibition surrounding the well. The antibacterial activity was expressed by the presence of zone of inhibition produced by the formulated gels when compared to plant extracts.[19,20]

RESULT AND DISCUSSION

The results of qualitative screening of phytochemical components in leaves of *A. scholaris* revealed the presence of alkaloids, coumarins, flavanoids, tannins and saponins were showed in table 1.

Table 1: Phytochemical components of different solvent leaves extracts of *A. scholaris.*

Phytochemical constituents	Chloro-form	Acetone	Methanol
Alkaloid	+++	--	+++
Anthraquonins	--	--	--
Coumarin	--	--	++
Flavanoids	--	++	++
Tannins	--	++	+++
Saponins	--	++	+++
Terpenoids	--	--	--
Cardiac glycosides	--	--	--
Steroids	--	--	--

Both the extract and formulated gel of leaves of *Alstonia scholaris* shows active varying degree of antimicrobial activity against test organism. The gel was found to be more active to all the test organisms compared to the extract.The antimicrobial activity of different plant extracts and formulated gels were performed and shown in figure 1 and table 2.The standard used was levofloxacin which have zone of inhibition with *Staphylococcus aureus*(24mm) and with *Klebsiella planticola* (26mm). In methanol gel, all the test organisms gave good susceptibility with zone of inhibition with *Staphylococcus aureus* (17mm) and with

Klebsiella planticola (19mm) which is higher when compared to other gels and extracts.

Table 2: Antimicrobial activity of different plant extracts and formulated gels.

Sl.no	Formulation	Name of organism	Time of Incubation	Zone of inhibition in (mm)	Inhibition zone with levofloxacin
1	Methanol extract	*Staphylococcus aureus*	24 hrs	13 mm	24 mm
		Klebsiella planticola	24 hrs	15 mm	26 mm
2	Methanol gel	*Staphylococcus aureus*	24 hrs	17 mm	24 mm
		Klebsiella planticola	24 hrs	19 mm	26 mm

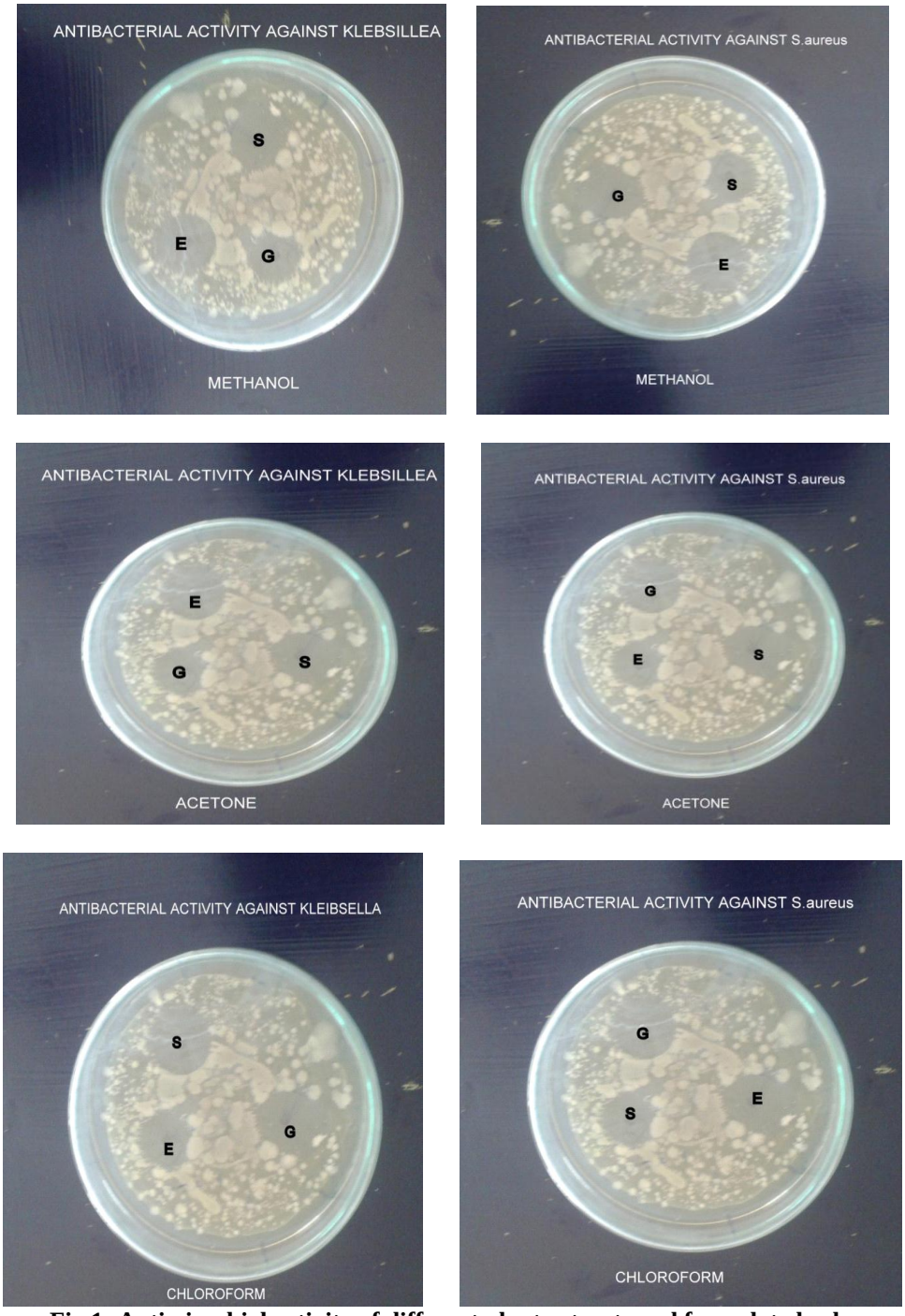

Fig 1: Antimicrobial activity of different plant extracts and formulated gels.

CONCLUSION

In this study, the fresh leaves of *Alstonia scholaris* were collected and dried. Different extracts of chloroform, methanol, ether and acetone were prepared. These extracts were used for the formulation of gel. Then antimicrobial activity of leaf extract and formulated gels

were done and shown in figure against *Staphylococcus aureus* and *Klebsiella planticola* by agar diffusion method. From the study it was observed that the formulated gels revealed greater antimicrobial activity as compared to its extract. Also from these formulated gels, methanolic extract of gel showed highest antimicrobial extract of gel showed highest antimicrobial activity as compared to chloroform, acetone and ether.

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