Assessment of Antibacterial Activities and Phytochemical Screening of Leaf and Fruit of *Solanum marginatum*

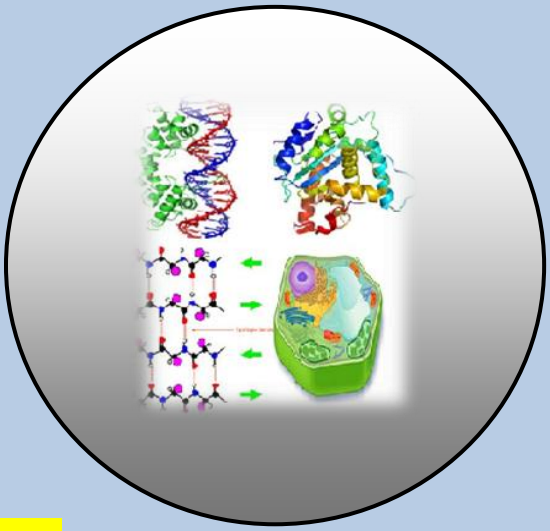
By

Misganaw T. Alemayehu Y. and Negera A.

ISSN 2319-3077 Online/Electronic
ISSN 0970-4973 Print

Journal Impact Factor: 4.275
Global Impact factor of Journal: 0.876
Scientific Journals Impact Factor: 3.285
InfoBase Impact Factor: 2.93
Index Copernicus International Value
IC Value of Journal 6.01 Poland, Europe

J. Biol. Chem. Research
Volume 32 (2) 2015 Pages No. 966-971



Journal of Biological and Chemical Research

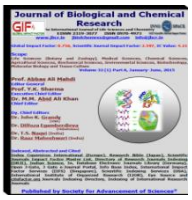
An International Peer Reviewed / Refereed Journal of Life Sciences and Chemistry

Indexed, Abstracted and Cited in various International and National Scientific Databases

Published by Society for Advancement of Sciences®

J. Biol. Chem. Research. Vol. 32, No. 2: 966-971, 2015
(An International Peer Reviewed / Refereed Journal of Life Sciences and Chemistry)
Ms 32/2/140/2015

ISSN 0970-4973 (Print)
ISSN 2319-3077 (Online/Electronic)



http:// www.sasjournals.com
http:// www.jbcr.in
jbiolchemres@gmail.com
info@jbcr.in

RESEARCH PAPER

Received: 26/10/2015 Revised: 11/11/2015 Accepted: 15/11/2015

Assessment of Antibacterial Activities and Phytochemical Screening of Leaf and Fruit of *Solanum marginatum*

Misganaw T. Alemayehu Y. and Negera A.

Chemistry Department, College of Natural Sciences, Chemistry Department, Jimma University, Ethiopia

ABSTRACT

Among medicinal plants that have vast array of medicinal uses by the community is Solanum marginatum. In this study the antibacterial activities of petroleum ether, chloroform, ethyl acetate and methanol extracts of both leaf and fruit of Solanum marginatum were investigated against Staphylococcus-aureus ATCC 25923, Escherichia coli ATCC 35218, Pseudomonas aeruginosa ATCC 27853 and Bacillus subtilis ATCC 6633 strains using agar-diffusion method. The fruit extracts showed antibacterial activity, with the methanol extract of the same part of the plant is the most active. Whereas, the leaf extract showed no remarkable activity against the four examined bacterial strains. The extracts were also subjected to preliminary phytochemical screening. The phytochemical screening revealed the presence of alkaloids, tannins, flavonoids, saponins and quinone in the most active extract of such plant

Key words: Antibacterial Activity, Phytochemical, Solanum Marginatum and Pharmacological activities.

INTRODUCTION

Natural products have been in use since ancient times as medicines, insecticides, natural dyes and spices, and the use of herbal remedies and dietary supplements. It was estimated that about 80% of all the world medicines are originally derived from plant sources, especially those found in tropical regions. However, many of the plants with in these often remote regions of the world have yet to be identified as species and only about 15% of the known angiosperm species in this region were examined for their medicinal potential. Therefore, there are most definitely a large number of plants derived medicines and other useful compounds that have to be discovered and characterized around the world (Negero et al., 2011**)** Studies by various researchers have proved that plants are one of the major sources for drug discovery and development. Plants are reported to have antimicrobial,

anticancer, anti-inflammatory, anti-diabetic, hemolytic and antioxidant properties etc. (Gordon ,et al., 2005)**.** In current day's medical and pharmaceutical advancement, microbes involve in the change of their metabolism and genetic structure to acquire resistant against the drugs used in the treatment of common infectious disease (Gaurav,et al., 2010). Among this wide range of medicinal plant parts are extracted as a raw drug and they possess varied medicinal properties. For example, in Ethiopia the origin of traditional medicinal plants are present in everywhere, *S. marginatum* is common ones. It is an important traditional medicinal plant for the treatment of wounds resulting from microbial infections which are one of the most common public health problems (Liu,et al., 2001).The common wound pathogens include: bacteria, fungi, protozoa and viruses among which the most common are bacteria hemolytic (Frankel ,et al., 1959). The quantity and quality of phytochemicals present in plant parts may differ from one part to another. In fact, there is lack of information on the distribution of the biological activity in different plant parts essentially related to the difference in distribution of active compounds (or active principles) which are more frequent in some plant parts than in others (Solomon ,et al., 2013).The presence of a phytochemical of interest may lead to its further isolation, purification and characterization. Then it can be used as the basis for a new pharmaceutical product. *Solanum marginatum* is introduced to part of the plants forested area. It has potential economic importance because its fruits are source of bioactive and different compounds used in the commercial production of sex hormones. Plants constituent a large reservoir of chemical substances that possess antimicrobial activity (Iwu, et al., 1999). In recent years, secondary metabolites previously with unknown pharmacological activities have been extensively investigated as a source of medicinal agents. Bioassay assisted extraction and identification of bioactive compounds and evaluation of the effect of bioactive compounds is such important (Sasidharan, et al.;2011). Since wound is one of the series problem in Ethiopia. But leaf and fruit of *solanum marginatum* are used to cure wound traditionally.This study was conducted as part of effort to validate the use of traditional medicine in Ethiopia, and investigate plants that are used locally, particularly for antimicrobial activity. This paper reports on phytochemicals, and antibacterial activity of the *S. marginatum*

MATERIAL AND METHODS

Plant Collection and preparation

The fresh plant leaves and fruits of *Solanum marginatum* were collected. The plant materials were air dried at room temperature under shade for seven days until all the water contents removed. The dried samples were ground separately into fine powder by using mortar and pestle

Extraction

About 339.7 g of fruits and 226.2 g of leaves powdered specimens were sequentially extracted with petroleum ether, chloroform, ethyl acetate and methanol using maceration technique. The extracts were filtered by using Whatman No-1 filter paper and the filtrates were concentrated using a rotary evaporator. Then the extracts were kept aseptically in desiccators until required for use.

Preparation of test samples

Test solutions were prepared by dissolving 100 mg of each of crude extract in 1mL of dimethyl sulfoxide (DMSO) to achieve final stock concentration of 100 mg mL^{-1} solution of test samples.

Preparation of fresh inoculums

Stock bacterial cultures were maintained at 4 °C on slants of nutrient agar. Active cultures for experiments were prepared by transferring a loop full of bacterial cells from the stock cultures to test tubes of nutrient broth that were incubated without agitation for 24 hrs at 37 °C. Cell suspensions of each organism were freshly prepared by transferring isolated colonies selected from a 24 hrs agar plate in to a broth.

Preparation of fresh inoculums

Stock bacterial cultures were maintained at 4 °C on slants of nutrient agar. Active cultures for experiments were prepared by transferring a loop full of bacterial cells from the stock cultures to test tubes of nutrient broth that were incubated without agitation for 24 hrs at 37 °C. Cell suspensions of each organism were freshly prepared by transferring isolated colonies selected from a 24 hrs agar plate in to a broth.

Micro Organisms

The following strains of bacteria were used in this study: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*)

Bioassay (Antibacterial Test) of the Crude Extracts

The antibacterial test was performed by using disc diffusion method. The bacteria isolates were grow in Petri dishes containing Mueller-Hinton agar using a sterile cotton swab. Then 6 mm diameter sterile discs (Whatman No 3 paper) were placed on the surface of the inoculated agar in Petri dishes, and 20 µl each test solutions were applie onto the discs. The same volume of DMSO and the standard drug (gentamicine, 30 mg) would be used as negative and positive controls respectively for comparision of antibacterial activity. After addition of test solutions on the discs, the extract would allowed to diffuse for 5-10 minutes and the plates will then be kept in an incubatator at 37 °C until 24 hrs. The antibacterial activities were evaluated by measuring the zone of growth inhibition surrounding the discs in millimeter with ruler.

Phytochemical Analysis of the Extracts

The crude extracts were screened qualitatively for the phytochemical contents following the procedure mentioned below(Oyedara et al.).

Test for Alkaloids (Wagner's reagent): A fraction of extract was treated with 3-5drops of Wagner's reagent 1.27 g of iodine and 2 g of potassium iodide in 100 ml of water and observed for the formation of reddish brown precipitate (or colouration)

Test for Flavonoids (Alkaline reagent test): 2 ml of extracts was treated with few drops of 20% sodium hydroxide solution. Formation of intense yellow colour, which becomes colorless on addition of dilute hydrochloric acid, indicates the presence of flavonoids.

Test for Quinones: A small amount of extract was treated with concentrated HCl and observed for the formation of yellow precipitate (or colouration).

Test for Saponins (Foam test): To 2 ml of extract was added 6 ml of distilled water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

Test for Tannins (Braymer's test): 2 ml of extract was treated with 10% alcoholic ferric chloride solution and observed for formation of blue or greenish colour solution.

RESULT AND DISCUSSION

Solanum marginatum is a species of nights shade known by the common names purple African nightshade and white-margined night shade.

And it is known on other continents as an introduced species and sometimes a weed. It is a hairy shrub growing up to two meters tall. The large, distinctive, gray-green leaves are wavy along the edges, woolly on the undersides. The fruit is a yellow berry up to 5 centimeters wide.


Figure1. Fruits, Steam bark and leaves of *Soalanum marginatum*

It has been used in Ethiopia as a traditional medicine for the treatment of different inflammatory disorders such as wound. It is also used for tanning activity around some areas and for the production of sex hormones industrially (Chizzali et al.). *Solanum marginatum* is one of several solanum species that contain solasodine. Solasodine is a poisonous alkaloid chemical compound that occurs in plants of the solanaceae family.

Mass of the extracts

Table 1. Mass of the extracts and percentage yields gained.

The plant parts socked	Mass of plant part used (g)	Solvent extracts	Mass of extracts gained (g)	% yield
Fruit	339.7	Methanol	3.91	1.15
		Ethyl acetate	2.73	0.80
		Chloroform	3.31	0.97
		Petroleum ether	3.12	0.92
Leaf	226.2	Methanol	5.16	2.28
		Ethyl acetate	3.18	1.41
		Chloroform	2.76	1.22
		Petroleum ether	2.89	1.28

Antibacterial Test of the Crude Extract

Table 2. Antibacterial activity of the fruit extracts.

Bacterial strain	Gram	Diameter of zone of Growth Inhibition (mm)					
		Petroleum ether extract	Chloroform Extract	Ethyl acetate extract	Methanol extract	G	DMSO
P. aeruginosa	+ve	NI	13	10	27	24	NI
E. coli	-ve	NI	NI	NI	NI	18	NI
S. aureus	+ve	NI	11	14	17	21	NI
B. subtilis	-ve	NI	NI	NI	NI	23	NI

key: NI = Not inhibitory, –ve = Gram negative, +ve = Gram positive, G = Gentamycin

Phytochemical Analysis of the Leaf and Fruit Extracts of *Solanum marginatum*

Table 3. Phytochemical analysis of the leaf and fruit extracts of S*olanum marginatum.*

No	Extracts	Part	Phytochemical testes				
			Alkaloids	Tannins	Saponins	Quinones	Flavonoids
1	Methanol	Fruits	++	+	+	+	+
2	Ethyl acetate		+	-	-	-	+
3	Chloroform		+	+	-	+	+
4	Petroleum ether		+	+	-	+	+
5	Methanol	Leaves	-	+	++	+	+
6	Ethyl acetate		-	-	-	-	-
7	Chloroform		-	+	-	-	-
8	Petroleum ether		-	-	-	-	+

KEY: ++ Present, + Slightly present, - Absent

DISCUSSION

In the present study, the antibacterial activity of the extracts was demonstrated by observing the clear inhibition zone on the plate. And compare the activity of the extracts and the control groups such as DMSO and standard antimicrobial gentamicine.

The bacterial activity of extracts of both fruit and leaf part of *Solanum marginatum* which were extracted successively with petroleum ether, chloroform, ethyl acetate and methanol were evaluated against four bacteria strains (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*). The extracts of the fruit part of the plant showed an activity with the methanol extract is the most active against *Pseudomonas aeruginosa* with the inhibition zone greater than that of the standard, gentamicine (table 2).Whereas, chloroform and ethyl acetate extracts of the same part of plant showed moderate activity against *P. aeruginosa* and *S. aureus*. However, all the extracts of leave part of *Solanum marginatum* didn't show an activity against the four bacteria test strains.

Following the remarkable activity of the extracts, phytochemical screenings of the extracts were performed (table 3). Indeed, the screening revealed that fruit part of the plant is rich in secondary metabolites including alkaloid, tannin, saponin, flavonoid and quinones. This could be the case for the better antibacterial activity of the fruit extracts than the leave part of *Solanu*m *marginatum*. The methanol extract contain all the secondary metabolites that have been screened and on the other hand, it showed a remarkable activity even better than the reference drug. Therefore, it is worth to identify the compound responsible for the better activity of the extract.

CONCLUSION

Fruit and leaf of *Solanum marginatum* were used as an herbal medicine for their wound healing. But in this study the leaf extracts of *Solanum marginatum* showed no activity against four examined bacterial strains. On the other hand, extracts of fruit of *Solanum marginatum* showed an activity two bacteria strains.

The methanol fruit extract dominantly inhabits *Pseudomonas aeruginosa* greater than the standard antimicrobial, gentamicin. So fruits of *Solanum marginatum* wound have medicinal importance but solvents used to extract the sample play a vital role. Therefore, it is important to use the fruit part of the plant for medicinal purpose provided that the dose and the solvent extract should be taken into account.

ACKNOWLEDGEMENTS

Jimma University, Chemistry Department and Biology Department are acknowledged for their overall support to accomplish this study.

REFERENCES

Chizzali, cornelia and Beerhues, ludger (**2012).** "phytoalexins of the pyrinae", Biphenyls and dibenzofurans, Beilstein J. Org. chem 8, 613-620.

Frankel, Gottfrid S (**1959)**. "The raison d'Etre of secondary plant substances", Science, 129,1416-1470

Gaurav Kumar, Loganathan Karthik, Kokati Venkata Bhaskara Rao (**2010).**" activity of aqueous extract of calotropis gigantea leaves – an in vitro study", International Journal of Pharmaceutical Sciences Review and Research , 4, 141.

Gordon MC, David JN(**2005).** "Bio diversity: A continuing source of novel drug leads", Pure Applied Chemistry, 77, 7-24.

Iwu, M.W., Duncan, A.R., Okunji, C.O. (**1999).**" New antimicrobials of plant origin", In: J. Janick (ed), Perspectives on New crops and new uses. Ashs Press,Alexandria VA. pp, 457-462.

Liu, x. f. F., Guan, Y. L., yang, D. Z., Li, Z. & Yao, K. D. **(2001) "** Antibacterial action of *solanum marginatum* and carboxilated *solanum marginatum"* , Jornal of Applied polymer science, 124 , 9 -11.

Negero Gemeda, Kelbessa Urga, Messay Getachew, Kissi Muddie, Frehiwot Teka, Ashenif Tadele, Hirut Lemma and Mulugeta Guta (**2011)**."Antibacterial activity of extracts from Myrtus *communis*. (ades) and dodoneae angustifolia l.f. (kitkita) using bioautography method, 10, 58-60.

Oyedara Omotayo O.(**2010).**" Evaluation of the in vitro antimicrobial activities and phytochemical compounds of leaf extracts of *lantana camara linn"*, Awolowo University, Ile Ife Osun State,Nigeria, , 31-33.

Sasidharan,Chen,Saravanan,Sundram ,Yoga Latha (**2011**)."Extraction, Isolation and Characterization of Biological compounds from plants' extract", Afr Journal Tradit Complement Altern Med.8(1),1-10

Solomon Charles Ugochukwu, Arukwe Uche I. and Onuoha Ifeanyi(**2013).**"Preliminary phytochemical screening of different solvent extracts of stem bark and roots of Dennetia tripetala G. Baker", Asian Journal of Plant Science and Research, 3, 10-13.

Corresponding author: Misganaw T. Alemayehu Y. and Negera A., Chemistry Department, College of Natural Sciences, Jimma University, Ethiopia

Email: yifrualemayehu@yahoo.com