Antibacterial Activity of Hibiscus Sabdariffa Extract and its Oil Against Selected Bacterial Isolates

Esraa Alawad[1], Mawada Babiker[2], Salma Alrasheed[3], NaserEldin Bilal[4]

[1,2,3,]*Student* , [4] *Professor, Department of Medical Microbiology*

Faculty of Medical Laboratory Sciences University of Khartoum-Sudan

Abstract- A total of 47 clinical bacterial isolates from different specimens (urine, sputum, wound swab,…etc) and 3 standard bacterial strains (S.aureus,E.coli, P.aeruginosa) were tested for their susceptibility to Hibiscus sabdariffa extract and it,s oil . This was carried out by the Cup Plate agar diffusion on Mueller- Hinton agar [MHA]. Hibiscus sabdariffa extract and it,s oil exhibited antibacterial activity against all types of tested bacteria both clinical and standard ones.

Keywords: Antibacterial Activity ,Hibiscus sabdariffa Extrac , Oil ,Bacterial Isolates.

I. INTRODUCTION

Herbalism (also herbology or herbal medicine) is the use of plants for medicinal purposes, and the study of botany for such use. Plants have been the basis for medical treatment through much of human history, and such traditional medicine is still widely practiced today. Modern medicine recognizes herbalism as a form of alternative medicine, as the practice of herbalism is not strictly based on evidence gathered using the scientific method. Modern medicine, does, however, make use of many plant-derived compounds as the basis for evidence-tested pharmaceutical drugs. Phytotherapy, and phytochemistry work to apply modern standards of effectiveness testing to herbs and medicines that are derived from natural sources. The scope of herbal medicine is sometimes extended to include fungal and bee products, as well as minerals, shells and certain animal parts.

1.1 Modern herbal medicine

The World Health Organization (WHO) estimates that 80 percent of the population of some Asian and African countries presently use herbal medicine for some aspect of primary health care. Pharmaceuticals are prohibitively expensive for most of the world's population, half of whom lived on less than $2 U.S. per day in 2002.[1] In comparison, herbal medicines can be grown from seed or gathered from nature for little or no cost.

Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies, including opium, aspirin, digitalis, and quinine. According to the World Health Organization, approximately 25% of modern drugs used in the United States have been derived from plants. At least 7,000 medical compounds in the modern pharmacopoeia are derived from plants.[2] Among the 120 active compounds currently isolated from the higher plants and widely used in modern medicine today, 80% show a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived.[3].

1.2 Hibiscus sabdariffa

Hibiscus sabdariffa L. (Hs), also known as roselle, is an ideal crop for developing countries as it is relatively easy to grow, can be grown as part of multi-cropping systems and can be used as food and fibre. In China the seeds are used for their oil and the plant is used for its medicinal properties, while in West Africa the leaves and powdered seeds are used in meals. Additionally, it is used in the pharmaceutical and food industries.

A limited number of reviews on Hibiscus sabdariffa have been conducted. Only one detailed review on the phytochemical, pharmacological and toxicological properties of Hibiscus sabdariffa[4] and two more focused, later reviews are available: One on the effectiveness of Hs in the treatment of hypertension[5] and another on the treatment of hypertension and hyperlipidemia. This review will focus not only on the phytochemistry and pharmacological properties of Hs in more detail, but also on economic-botanical aspects of Hs, its scientific applications and translational research.

1.3 Botanical description

The genus Hibiscus (Malvaceae) includes more than 300 species of annual or perennial herbs, shrubs or trees. Hs (syn.: Abelmoschus cruentus (Bertol.) Walp.,Furcaria sabdariffa Ulbr., Hibiscus cruentusBertol., Hibiscus fraternus L., Hibiscus palmatilobus Baill. and Sabdariffa rubra Kostel is commonly known as roselle, hibiscus, Jamaica sorrel or red sorrel (English) and in Arabic, karkadeh. Its native distribution is uncertain, some believe

that is from India or Saudi Arabia, while Murdock showed evidence that Hs was domesticated by the black populations of western Sudan (Africa) sometime before 4000 BC. Nowadays, it is widely cultivated in both tropical and subtropical regions including India, Saudi Arabia, China, Malaysia, Indonesia, The Philippines, Vietnam, Sudan, Egypt, Nigeria and México[6].

There are two main varieties of Hs, the first being Hs var. altissima Wester, c ultivated for its jute-like fibre and the second is Hs var. sabdariffa. The second variety includes shorter bushy forms, which have been described as races: bhagalpuriensi, intermedius, albus and ruber. The first variety has green, red-streaked, inedible calyces, while the second and third race have yellow-green edible calyces (var. ruber) and also yield fibre.

II. OBJECTIVES

To investigate the antibacterial activity oh hibiscus sabdariffal extracts and its oil against selected clinical bacterial isolates and standard bacterial strains of ATCC types.

III. MATERIAL AND METHODS

3.1 Collection, identification of clinical bacterial isolates

The samples were collected during the period of time 1/3/2017 to 30/3/2017.

Selected forty seven Gram positive and Gram negative bacteria were isolated from different clinical specimens including urine, wound, sputum, ear swab, fluid aspirate, from Soba University Teaching Hospital, Khartoum Teaching Hospital, and National Public Health Laboratory.

The isolated bacteria were fully identified by Gram stain, catalase test, coagulase, oxidase, indole, citrate utilization, urease tests, in addition to Kilgler iron agar.

3.2 Plant material:

Sample of Hibiscus sabdariff were obtained from Omdurman market, the only place where the seeds were available.

3.2.1 Preparation of crude extract of Hibiscus sabdariff:

Extraction was carried out according tothe method described by Sukhdev[7]:

1-Ethanolic extract preparation:

240g of the Hibiscus sabdariffcycles sample was grounded using mortar and pestle and extracted by soaking 80% ethanol for five days with daily filtration and evaporation. Solvent was evaporator apparatus and the extract was allowed to air dry till complete dryness. Yield percentage was calculated as followed:

(Weight of extract/ weight of sample)* 100

(120/240)*100= 50%

2. N-hexane extract preparation:

900 g of Hibiscus sabdariff seeds was coarsely powdered using mortar and pestle. Coarsely sample was successively extracted n-hexane using soxhelt extractor apparatus. Extraction carried out for about four hours till the colour of solvents at the last siphoning time returned colorless. Solvents were evaporated under reduced pressure using rotary evaporator apparatus. Finally extracts allowed to air in petri dish till complete dryness and the yield percentage were calculated as followed:

(Weight of extract obtained / weight of plant sample) * 100

(50/ 900)*100= 5.6%

3. Aqueous extract preparation:

200 g of Hibiscus sabdariff sample were soaked in 1000 ml of distilled water for 24 hours. And then filter the solution extracted from the process of soaking and save in the refrigerator and then insert the water extract of the device Freeze Dryer Modulyo. The aqueous extract is dried by the Freeze Dryer to get it in the form of a powder.

3.3 Testing of extracts for antibacterial activity against standard bacteria and clinical isolates:

Cup- plate agar diffusion method:

Cup plate agar diffusion method was adopted with some minor modifications to assess the antibacterial activity of prepared extract 0.2ml of bacterial suspension (standard and clinical isolates) were taken with automatic Ependorff pipettes using sterile tips and added to twenty ml of molten Mueller Hinton media and mixed and poured in sterile Petri dish. The media were allowed to set and solidify for 20 minutes, make wells using sterile Cork borer of 10 mm diameter. Alternated cups were filled with 0.1 ml of different concentrations of ethanolic extract, aqueous extract and the oil (100mg/dl, 50mg/dl, 25mg/dl, 12.5mg/dl, 6.25mg/dl) using automatic pipettes. Allowed to diffuse at room temperature for 30 min then the plates were incubated aerobically in an incubator in upright position at 370c for 18 hours. The diameters of the

resultant growth inhibition zones were measured in mm. the inhibition zones with diameter less than 12 mm were considered as having no antibacterial activity.[8

The standard strains used in this study were: S.aureus ATCC 25923, E.coli ATCC 25922 and P.aeruginosa ATCC 27853

IV. RESULTS

Table (1):Types and numbers of clinical bacterial isolates:

bacterial isolates	Number (%)
P.aeruginosa	(29)
E.coli	(27)
K.peumoniae	(21)
S.aureus	(19)
P.mirabilis	(2)
Total	(100)

Table (2): The number and types of samples collected:

Type of specimen	Number (%)
Urine	(53)
Wound swab	(27)
sputum	(14)
Aspirate fluid	(2)
Ear swab	(2)
Total	(100)

This experimental study was conducted from March to April in 2017.

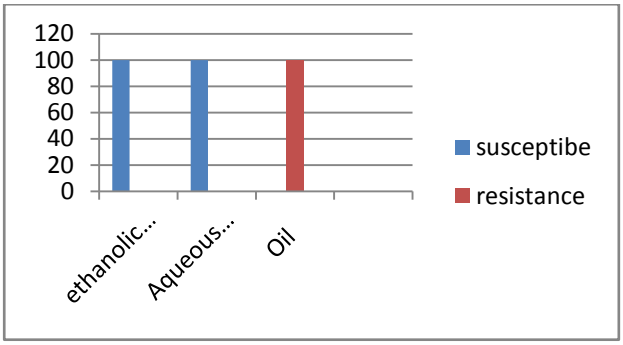

Fig (1): Percentage of extracts activity against E.coli.

The ethanolic one and aqueous extracts of Hibiscus sabdariffexhibited 100% activity against S.aureus, E.coli, P.aeruginosa, P.mirabilis, while in K.peumoniae exhibited (90%) as shown in the figures (1,2,3,4 and 5).

The oil extract of Hibiscus sabdariffcereal showed no activity against all clinical bacterial isolates as shown in the figures (1, 2, 3, 4 and 5).

The ethanolic extract and aqueous extract had the same activity against all clinical bacterial isolates as shown in the figures (1, 2, 3, 4 and 5).

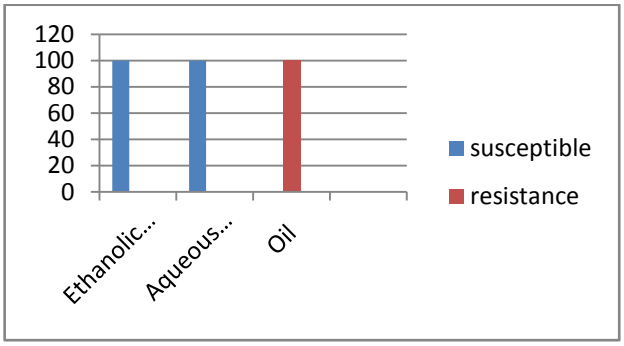

Fig (2): Percentage of extracts activity against S.aureus.

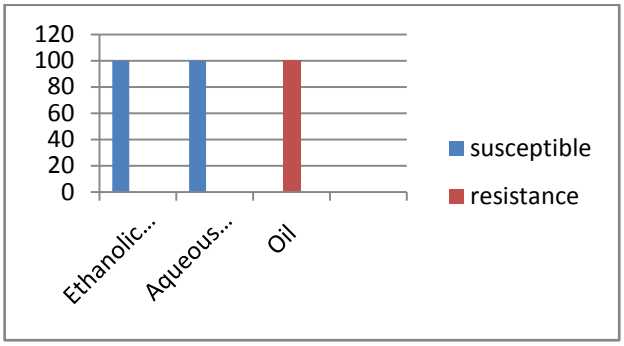

Fig (3): Percentage of extracts activity against P.mirabilis.

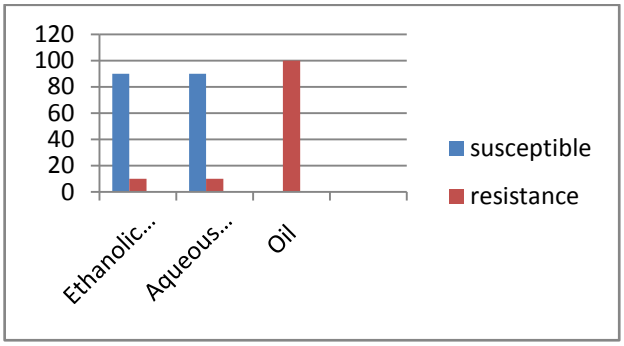

Fig (4): Percentage of extracts activity against K.peumoniae.

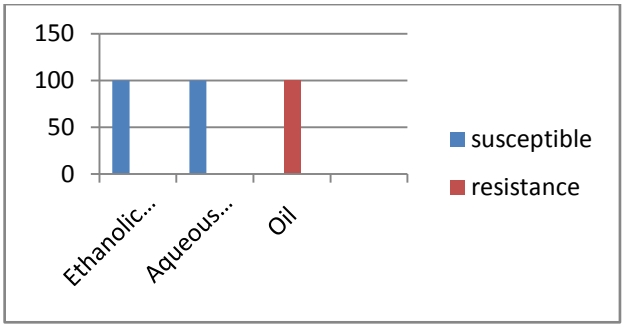

Fig (5): Percentage of extracts activity against P.aeruginosa.

The highest mean diameter of the growth inhibition zone in mm (MDIZ) 0f Hibiscus sabdarifffor S.aureus was 19 mm at concentration 100mg/dl 0f aqueous extract, 18.8 mm at concentration 100mg/dl of ethanolic extract, while the lowest MDIZ was (0 mm) at concentrations (25mg/dl, 12.5mg/dl and 6.25mg/dl) for ethanolic and aqueous extracts.

The highest MDIZ was 17.7 mm for E.coliat 100mg/dl of ethanolic extract and 14.6 mm at 100mg/dl of aqueous extract, the lowest MDIZ was (0 mm) at (25mg/dl, 12.5mg/dl and 6.25mg/dl) for both ethanolic and aqueous extracts.

The highest MDIZ for K.peumoniae was 15.7 mm at 100mg/dl of ethanolic extract, 15.4 mm at 100mg/dl of aqueous extract, the lowest MDIZ was (0 mm) at (25mg/dl, 12.5mg/dl and 6.25mg/dl) for both extracts.

The highest MDIZ for P.mirabilis15 mm at 100mg/dl for both ethanolic and aqueous extracts, while all other concentration of extracts showed no activity against it.

The highest MDIZ for P.aeruginosa was 20.9 mm at 100mg/dl of ethanolic extract and 20.4 mm at 100mg/dl of aqueous extract, while the lowest MDIZ was (0 mm) at (12.5mg/dl and 6.25mg/dl) for both extracts.

The oil of Hibiscus sabdariff cereal failed to show any activity at all concentrations for all clinical bacterial isolates and standard bacterial strains. (Table 4).

Table (3): Antibacterial activity of the *Hibiscus sabdariffa* extracts and oil against standard ATCC strains:

ATCC strains	Ethanolic extract					Aqueous extract					0il extract				
	100 mg/dl	50mg/dl	25mg/dl	12.5 mg/dl	6.25 mg/dl	100 mg/dl	50mg/dl	25mg/dl	12.5 mg/dl	6.25 mg/dl	100 mg/dl	50mg/dl	5dl 25mg/dl	12.5 mg/dl	6.25 mg/dl
S. aureus	S	S	S	-	-	S	S	-	-	-	-	-	-	-	-
E.coli	S	S	-	-	-	S	S	S	-	-	-	-	-	-	-
P.aeruginosa	S	S	-	-	-	S	-	-	-	-	-	-	-	-	-

Table (4): The mean diameter of growth inhibition zone (MDIZ) in millimeter (mm) of *Hibiscus sabdariffa* extracts and oil against clinical isolates:

Clinical isolates	Ethanolic extract					Aqueous extract					0il extract				
	100 mg/dl	50mg/dl	25mg/dl	12.5 mg/dl	6.25 mg/dl	100 mg/dl	50mg/dl	25mg/dl	12.5 mg/dl	6.25 mg/dl	100 mg/dl	50mg/dl	25mg/dl	12.5 mg/dl	6.25 mg/dl
S.aureus	18.8	14.2	0	0	0	19	12.6	0	0	0	0	0	0	0	0
E.coli	17.7	11.3	0	0	0	14.6	9.3	0	0	0	0	0	0	0	0
K.pneumoiae	15.4	12.5	0	0	0	15.7	10	0	0	0	0	0	0	0	0
P.mirabilis	15	0	0	0	0	15	0	0	0	0	0	0	0	0	0
P.aeruginosa	20.4	15.3	7	0	0	20.9	14.8	6.5	0	0	0	0	0	0	0

V. DISCUSSION

Hibiscus sabdariffa was found to be active against S.aureus, K.peumoniae and P.aeruginosa. These finding are in agreement withKeh-sen Liu, who tested invitro inhibitory effect of roselle calyx on the growth of methicillin-resistant Staphylococcus aureus, Klebsiella

pneumoniae andPseudomonas aeruginosa. Roselle calyx extract effectively inhibited the growth of all tested bacterial pathogens. [9]

The aqueous extract of Hibiscus sabdariffawas tested for antibacterial activity against S.aureus, E.coli, K.peumoniae and P.aeruginosa. The result obtained are in consistent with Olaleye, Mary Tolulope who found that aqueous-methanolic extract of H. sabdariffa exhibited antibacterial activities against Staphylococcus aureus, Escherichia coli, Klebsiellapneumoniae and Pseudomonas aeruginosa. [10]

The MIDIZ 17.7mm at100mg/dl for E.coli which we isolated from clinical samples was similar to the result of Marjorie Fullerton, who found thatthe antimicrobial activity of sorrel on Escherichia coli O157:H7 isolates from food.The mean zone of inhibition for the sorrel extract was 12.66 mm for 10%, 10.75 mm for 5%, and 8.9 mm for 2.5%. The highest inhibition zone was (11.16 mm). [11]

The results of E.coli and S.aureusare concise and consistent with the results obtained by Mohamad Bokaeian, who examined the evolution of antimicrobial activity of flower extract of Hibiscus sabdariffal against Escherichia coli and Staphylococcus aureus isolated from the urinary tract infection. The result showed

The Hibiscus sabdariffal extract effectively inhibited the growth of all tested bacterial pathogens. [12]

VI. CONCLUSIONS:

The aqueous extract of Hibiscus sabdariffa was active against all clinical bacterial isolates. Hibiscus sabdariffa inhibited the growth of all isolated bacteria and possess some active Phytochemicals that can inhibit the growth of bacteria.[13]

VII. RECOMMENDATIONS:

In vivo investigation to evaluate the antibacterial activity of Hibiscus sabdariffa extract on experimental animals.

Testing the Hibiscus sabdariffa extract on a larger number of samples and on other types of bacteria, fungi as well.

Acknowledgments:

The author wish to acknowledge the staff of microbiology department at the faculties of Pharmacy and Medical Laboratory Sciences , University of Khartoum.

REFERENCES

[1] Edgar J. DaSilva; Elias Baydoun; Adnan Badran "Biotechnology and the developing world". Electronic Journal of Biotechnology. (2002). 5 (1). doi:10.2225/vol5-issue1-fulltext-1. ISSN 0717-3458.

[2] Interactive European Network for Industrial Crops and their Applications "Summary Report for the European Union".(2000–2005). QLK5-CT-2000-00111. Archived from the original on 2013-12-22. [www.ienica.net/reports/ienicafinalsummaryreport2000-2005.pdf Free full-text].

[3] Fabricant DS, Farnsworth NR "The value of plants used in traditional medicine for drug discovery". Environ. Health Perspect. (March 2001). 109 (Suppl 1): 69–75. doi:10.1289/ehp.01109s169. PMC 1240543 . PMID 11250806.

[4] Ali, Al Wabel, & Blunden,Phytochemical, pharmacological and toxicological aspects of Hibiscus sabdariffa L.: a review. Phototherapy Research.2005 May;19(5):369-75.

[5] Wahabi, Alansary, Al-Sabban, & Glasziuo,The effectiveness of Hibiscus sabdariffa in the treatment of hypertension: a systematic review.

[6] Phytomedicine. 2010 Feb;17(2):83-86.

[7] Chewonarin.,Dung., Eslaminejad , Zakaria,Ismail,Mahran., et al. Composition of the Seed Oil of Hibiscusabelmoschus L. (Malvaceae) Growing in Vietnam. Journal of Essential Oil Research. July 1999 11(4):447-452 •

[8] 7- Sukhdev.S. H;Suman. P.S.K; Gennaro. L and Dev. D.R. Extraction technologies for medicinal and aromatic plants. United Nation Industrial Development Organization and the International Center for Science and High Technology. (2008) pp116.

[9] Srinivasan. D. Sangeetha Nathan, Suresh, T, Lackshmanaperumalsamy, P. J. Antimicrobial activity of certain Indian medicinal plants used in folkoric medicine. Ethnopharmacol.2001;74,217-220.

[10] Keh-sen Liu, antibacterial activity of roselle calyx and protocatechuic acid against pathogenic bacteria. Phototherapy Research. November 2005. 19 (11): 942–945.

[11] Olaleye, Mary Tolulope, antimicrobial activity and cytotoxicity of H. sabdariffa. Journal of Medicinal Plants Research. August 2007 1(1): 9-13,.Available online at http://www.academicjournals.org/JMPR © 2007 Academic Journals.

[12] Marjorie Fullerton, Janak Khatiwada, Jacqueline U. Johnson, Shurrita Davis, and Leonard L. Williams. Journal of Medicinal Food. August 2011, 14(9): 950-956.

[13] Mohamad Bokaeian, antimicrobial activity of flower extract of Hibiscus sabdariffal against antibiotic-resistant Escherichia coli and Staphylococcus aureus that isolates from the urinary tract infection, International Journal of Advanced Biological and Biomedical Research. February 2014. 2(2): 433-439.

[14] Badreldin H. Ali, Naser Al Wabel, Gerald Blunden. Phytochemical, pharmacological and toxicological aspectsofHibiscus sabdariffaL.Phototherapy Research. May 2005. 19(5): 369–375