Advancement in Medicinal Plant Research
Vol. 3(1), pp. 18-22, February 2015
ISSN: 2354-2152
Full Length Research Paper

Antimicrobial potential of methanolic extracts of *Hibiscus sabdariffa* and *Ricinus communis.*

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Accepted 4 February, 2015

ABSTRACT

Antimicrobial resistance threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi. Patients with infections caused by drug-resistant microorganisms are generally at increased risk of worse clinical outcomes and death, and consume more healthcare resources. This study was carried out to evaluate the antimicrobial potential of leaves and seeds methanolic extracts of the medicinal plants *Hibiscus sabdariffa* and *Ricinus communis* against standard microbial strains include Gram-positive bacteria, Gram-negative bacteria and fungal strains. The agar well diffusion technique was carried out to perform the antimicrobial activity of the candidate plants. Leaves from methanolic extracts of *H. sabdariffa* was found to be more active against Gram-positive bacteria (*Bacillus subitilis* NCTC: 8236 and *Staphylococcus aureus* ATCC: 25923), as well as Gram-negative bacteria (*Escherichia coli* ATCC: 25922, *Pseudomonas aeruginosa* ATCC: 27853, *Klebsiella pneumoniae* ATCC: 53657 and *Proteus vulgaris* ATCC: 6380) than leaves methanolic extracts of *R. communis*. The leaves from methanolic extracts of *H. sabdariffa* obtained an intermediate antifungal activity against two reference fungal strains (*Candida albicans* ATCC: 7596 and *Aspergillus niger* ATCC: 9765). The seeds from methanolic extracts of *R. communis* and *H. sabdariffa* did not show any activity against both bacterial and fungal reference strains examined. In the present study, methanolic leaves extracts of *H. sabdariffa* revealed that the selected plant had a significant potential effect to inhibit the growth of both bacterial and fungal strains.

Keywords: *Ricinus communis*, *Hibiscus sabdariffa*, antimicrobial potential, methanolic extracts, *Escherichia coli*, seeds extract.

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INTRODUCTION

Medicinal plants have been used as sources of remedies in virtually all cultures. During the last decade, the use of traditional medicine has expanded globally and is gaining popularity. It has continued to be used not only for primary health care of the poor in developing countries, but also in countries where conventional medicine is predominant in the national health care system (Hailu et al., 2005). Nature has been a huge source of antimicrobial and other medicinal product since pre - historic times, the importance of using herbal products in the treating of various human diseases are not limited. It is obvious that the plant kingdom harbours inexhaustible sources of active ingredients that are valuable in management of many serious and complicated diseases. Therefore, medicinal plants are significant for the study of their conventional uses through the confirmation of their pharmacological effects (Rabia and Asghari, 2012). The global emergence of antimicrobials resistance is fueled by the wide spread use of broad-spectrum antimicrobial agents, creating continuous selective pressure, and by lapses in infection control, which facilitate transmission of resistant pathogenic microorganisms (Bonten et al., 2001). *Hibiscus sabdariffa* (Linn.) is a species belonging to the family *Malvaceae* locally known as "Karkadi" it is

native to the old world tropics, an annual or perennial subshrub, about 2 to 2.5 m tall (Copley, 1975; Leung and Foster, 1996). *H. sabdariffa* plant has been used in folk medicine as a diuretic, laxative, and treatment for cardiac and nerve diseases and cancer (Chewonarin et al., 1999). The heated leaves are applied to cracks in the feet and on boils and ulcers to speed maturation. A lotion made from leaves is used on sores and wounds; although its leaves and seeds have well documented hypotensive effects (Haji and Haji, 1999; Bako et al., 2010). Phytochemically, the plant contains flavonoids and tocopherol (Mohamed et al., 2007). Many studies have been carried out in different parts of the world to evaluate the antimicrobial activity of various parts extracts of *H. sabdariffa*. Most of these studies revealed that the plant has a potent antimicrobial properties (El-Kamali and Mohammed, 2006; El manama et al., 2011). *Ricinus communis*, locally known as"Khirwea" belonging to the family *Euphorbiaceae* is a plant that can vary greatly in its growth habit and appearance (Christopher, 1996). The seeds castor oil has many uses in medicine and other applications. An alcoholic extract of the leaves was shown, in laboratory rats, to protect the liver from damage from certain poisons (Kalaiselvi et al., 2003; Joshi et al., 2004). *R. communis* seeds and leaves contain terpenoids and a tocopherol-related compound in its aerial parts. Different extracts of the leaves and seeds of *R. communis* has showed promised antimicrobial effect against pathogenic bacteria and fungi (Islam et al., 2010; Sabina et al., 2009; El-Sharif and El-Rofaei, 2011; Rabia and Asghari, 2012; Manik et al., 2013). The present study was aimed to investigate the antimicrobial potential of both leaves and seeds from methanolic extracts of these medicinal plants *R. communis* and *H. sabdariffa* respectively, and to determine their minimum inhibitory concentrations (MIC) on the test microorganisms.

MATERIALS AND METHODS

Collection of plant materials

Leaves and seeds of *H. sabdariffa* and *R. communis* were collected from SHAMBAT Area-Khartoum North, Sudan. They were authenticated by Professor Hatil Hashim EL-Kamali, Professor of Botany, Faculty of Science and Technology, Omdurman Islamic University, Omdurman, Sudan. The work has been carried out at Microbiology laboratory at Medicinal and Aromatic Plants Research Institute (MAPRI). Voucher specimens were deposited at the herbarium of the institute.

Preparation of crude plant extracts

Each of the coarsely powdered plant material (50 g) was exhaustively extracted for 20 h with methanol by Soxhlet apparatus. The extracted plant material was then air-dried, repacked in the Soxhlet and exhaustively extracted with methanol. The methanolic extract was filtered and evaporated under reduced pressure again using Rota-vapor. Each residue was weighed and the yield percentage was determined.

Test microorganisms

Eight different standard strains were used for testing antimicrobial activity include two Gram- positive bacterial species: (*Bacillus subtilis* NCTC 8236, *Staphylococcus aureus* ATCC 25923), four Gram- negative bacterial species (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 53657, *Proteus vulgaris* ATCC 6380 and *Pseudomonas aeruginosa* ATCC 27853) and two fungal species (*Candida albicans* ATCC7596 and *Aperugillus niger* ATCC 9763). The standard strains were obtained from National Collection of Type Culture (NCTC), Colindale, England and American Type Culture Collection (ATCC), Rockville, Maryland, USA.

Culturing of microorganisms

The test microorganisms were inoculated on blood agar, nutrient agar plates and sabouraud's dextrose agar, they were incubated aerobically and the obtained growth were then purified by streaking on plates containing the appropriate selective and differential culture media, mannitol salt agar and macConkey's agar. The purified microorganisms were maintained by periodically sub culturing and preserved at 4°C prior to use (Collee and Marr, 1996).

Preparation of inoculum

1 ml aliquots of a 24 h broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 h. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 10^8 to 10^9 CFU/ml. The suspension was stored in the refrigerator at 4°C till used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique. Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37°C for 24 h. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension. Each time a fresh stock suspension was prepared; all the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

Testing of extracts for antimicrobial activity

The cup-plate agar diffusion method (Kavanagh, 1972) was adopted with some minor modifications to assess the antibacterial and antifungal activity of the prepared extracts. 1 ml of the standardized bacterial and fungal stock suspension 10^8 to 10^9 CFU/ml were thoroughly mixed with 100 ml of molten sterile nutrient agar which was maintained at 45°C. 20 ml aliquots of the inoculated nutrient agar were distributed into sterile Petri-dishes. The agar was left to set and in each plate 4 cups (10 mm in diameter) were cut using a sterile cork borer (No. 4) and agar discs were removed. Alternate cups were filled with 0.1 ml sample of each extracts using automatic microlitre pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the

Table 1. Antimicrobial activity of candidate methanolic extracts against standard microorganisms.

Plant name	Part used	Yield %	Conc. used/ml (mg)	Standard microorganisms /MDIZ mm							
				E.c	***Ps.a***	***Kl.p***	***P.v***	***B.s***	***S.a***	***C.a***	***Asp.n***
R. communis	Leaves	5.8	100	15	15	16	13	15	20	14	15
	Seeds	9.8	100	-	-	-	-	-	-	-	-
H. sabdariffa	Leaves	11	100	20	22	22	19	21	26	24	17
	Seeds	29	100	-	-	-	-	-	-	-	-

Key: Standard microorganisms (*E.c: Escherichia coli, Ps.a: Pseudomonas aeruginosa, Kl.p: Klebsiella pneumoniae, P.v: Proteus vulgaris*, *B.s: Bacillus subtilis, S.a: Staphylococcus aureus, C.a*: *Candida albicans* and *Asp.n*: *Apergillus niger*). MDIZ: Mean diameter inhibition zone. (-): No inhibition zone.

upright position at 37°C for 18 h. Two replicates were carried out for each extract against each of the test organisms. After incubation the diameters of the resultant growth inhibition zones were measured, averaged and the mean values were tabulated, as well as, the antimicrobial activity of reference antibiotics viz: Ampicillin, Tetracyclin, Nystatin and Clotrimazole was determined in order to compare their activity with the activity of candidate extracts.

Determination of minimum inhibitory concentration (MIC)

The principle of the agar plate dilution is the inhibition of growth on the surface of the agar by the plant extracts incorporated into the medium. Plates were prepared in the series of increasing concentrations of the plant extract. The bottom of each plate was marked off into 4 segments. The organisms tested were growing in broth over night to contain 10^8 CFU/ml. Loop-full of diluted culture is spots with a standard loop that delivers 0.001 ml on the surface of segment. The endpoint (MIC) is the least concentration of antimicrobial agent that completely inhibits the growth. Results are reported as the MIC in mg/ml.

RESULTS AND DISCUSSION

The mean diameters of inhibition zone (MDIZ) produced by candidate extracts on standard microorganisms are presented in Table 1. On the other hand, Table 2 shows antimicrobial activity of the reference chemotherapeutic drugs against the test microorganisms. The results were interpreted as sensitive, intermediate and resistant. Based on the results of Table 2, plant extracts resulting in 15 mm or more MDIZ are considered to be active and those resulting in less than 15 mm are inactive (Cruickshank et al., 1975). The minimum inhibitory concentration (MIC) of the methanolic extract of *H. sabdariffa* leaves against standard microorganisms has been shown in Table 3. It is clear from Table 1 that the leaves from methanolic extract of *R. communis* showed high activity only against *Staphylococcus aureus*, whereas it has an intermediate activity against *E. coli*, *Pseudomonas aeruginosa, Klebsiella pneumoniae, Bacillus subitilis*, and *Aspergillus niger*. The same extract was found to be inactive against *Proteus vulgaris* and *Candida albicans*. In contrast to study of Rabia and Asghari (2012) methanolic leaves extract from *R. communis* was found to be highly active against both Gram-positive and Gram-negative bacteria.

The seeds of methanolic extract from *R. communis* did not show any activity against both bacterial and fungal reference strain examined in the present study. This finding is in agreement with that study of El-Sharif and El Rofaei (2011) in which the seeds methanolic extract from *R. communis* exhibited high antimicrobial activity against all tested microorganisms which include Gram-negative, Gram-positive bacteria, and tow fungal species. The leaves from methanolic extract of *H. sabdariffa* revealed high activity against *Staphylococcus aureus* (26 mm), *Pseudomonas aeruginosa* (22 mm), *Klebsiella pneumoniae* (22 mm), *Bacillus subitilis* (21 mm), *Escherichia coli* (20 mm), *Proteus vulgairs* (19 mm), *Candida albicans* (24 mm) and *Aspergillus niger* (17 mm). The antimicrobial activity of *H. sabdariffa* leaves methanolic extract may be due to the presence of polyphenolic compound such as flavonoid. This finding is agreed in points with study of Elmanam et al. (2011) in Palestine. The seeds methanolic extract of *H. sabdariffa* had no activity against the test microorganisms in our study. This finding is in agreed with the recent study in Ivory Coast (Lessoy et al., 2012) which revealed that the seed oil of *H. sabdariffa* exhibited high antimicrobial activity against *S. aureus*, *Aspergillus fumigatus* and *Trychophyton mentagrophytes*. In this study, *S. aureus* was found to be the most sensitive organism being inhibited by the two extracts, while *P. vulgaris* showed the lowest susceptibility being inhibited by only one extract. Therefore, the Gram-positive bacteria were more sensitive than the Gram-negative bacteria. It is known that the pattern of inhibition varied with the plant part, the solvent used and the microorganism that tested. The comparison of observations, provided in Tables 1 and 2 illustrated that the leaves methanolic extract of *R. communis* showed high activity against *S. aureus* (20 mm) which is almost similar to 20 µg/ml Ampicillin and 5 to 10 µg/ml Tetracyclin. *R. communis extract* inhibited *K. pneumoniae* (16 mm) which is less than activity of 20 µg/ml Ampicillin. The same extract was found to be moderately active against *A. spergillus niger* (15 mm) which is lower than activity of 25 mg/ml Nystatin and 20

Table 2. Activity of reference antimicrobial drugs on the standard microorganisms.

Antimicrobial drug	Conc. used (µg/ml)	Standard microorganisms /MDIZ mm							
		E.c	***Ps.a***	***Kl.p***	***P.v***	***B.s***	***S.a***	***C.a***	***Asp.n***
Ampicillin	40	18	-	18	-	15	25	ND	ND
	20	16	-	15	-	14	20	ND	ND
	10	13	-	13	-	13	18	ND	ND
	5	-	-	12	-	12	15	ND	ND
Tetracyclin	40	24	16	27	16	23	31	ND	ND
	20	19	13	25	-	21	27	ND	ND
	10	-	12	21	-	20	25	ND	ND
	5	-	-	18	-	18	17	ND	ND
Nystatin	25 mg	ND	ND	ND	ND	ND	ND	14	26
Clotrimazole	20 mg	ND	ND	ND	ND	ND	ND	24	34

ND: Note determined.

Table 3. MIC of methanol extract of *H. sabdariffa* against standard microorganisms.

Part used	Standard microorganisms (mg/ml)							
	E.c	***Ps.a***	***Kl.p***	***P.v***	***B.s***	***S.a***	***C.a***	***Asp.n***
Leaves	12.5	12.5	25	12.5	6.25	12.5	6.25	25

mg/ml Clotrimazole, while it was inactive against *Candida albicans* (14 mm). The leaves methanol extract of *H. sabdariffa* exhibited highest activity (26, 22, 22, 21 and 20 mm) against *S. aureus, P. aeruginosa, K. pneumoniae, B. subitilis* and *E. coli,* respectively, which are higher than 40 µg/ml Ampicillin. The leaves methanolic extract of *H. sabdariffa* extract was found to be moderately active against *C. albicans* (15 mm) which is more than activity of 25 mg/ml Nystalin and lower than activity of Clotrimazole 20 mg/ml. The same extract also inhibited *A. niger* (17 mm) which is lower than activity of 25 mg/ml Nystatin and 20 mg/ml Clotrimazole.

CONCLUSION

Leaves methanolic extract of *H. sabdariffa* was found to be having potent effect against tested microorganisms. Seeds of methanol extracts of both *R. communis* and *H. sabdariffa* did not show any activity against examined microorganism. Generally, the most sensitive organisms in this study were *Staphylococcus aureus* and *Candida albicans* inhibited by the two methanolic plants extracts, while the least sensitive organism was *Proteus vulgaris,* which inhibited by methanolic extract of *H. sabdariffa*. The results may provide valuable information and elucidate the effective role of these plants as a useful topic for the clinical evaluation and development of commercial curative extracts.

RECOMMENDATION

Methanolic extract of *H. sabdariffa* revealed promising biological activities and could form a good basis for its selection for further investigation in order to develop new natural bioactive compounds. Regarding this activity, more clinical trials should be carried out to verify its in vivo interaction.

ACKNOWLEDGEMENTS

The authors greatly acknowledge the support and facilities of Medicinal and Aromatic Plants Research Institute and for permission to perform the laboratory work. Particular thank to all Staff of Microbiology Department. The authors also indebted to Professor Hatil Hashim EL-Kamali, Professor of Botany, Faculty of Science and Technology OIU, for the authentication of the studied plant.

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Citation: Abd-Ulgadir KS, Suliman SI, Zakria IA, Hassan NA, 2015. Antimicrobial potential of methanolic extracts of *Hibiscus sabdariffa* and *Ricinus communis*. Adv Med Plant Res, 3(1): 18-22.