First report about pharmaceutical properties and phytochemicals analysis of *Rosa abyssinica* R. Br. ex Lindl. (Rosaceae)

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Abstract: *In vitro* antimicrobial efficacy of seven solvent extracts from leaves and hips of Saudi Arabian weed *Rosa abyssinica* against a variety of human pathogenic bacteria and *Candida* species have been evaluated using well diffusion methods. Phytochemicals present in the leaves and hips of *Rosa abyssinica* has been characterized using Gas Chromatogram Mass spectrometry analysis. The extracts comparative efficacy against tested microbes gained from the fresh and dry leaves exhibited more prominent activity than fresh and dry hips. The methanol, chloroform, petroleum ether, acetone and diethyl ether extracts have a greater lethal effect on pathogenic microbes than hot water extracts, while cold-water extracts showed no activity. Twenty-four phytochemicals have been characterized from ethanol extract of the leaves of *Rosa abyssinica* and fifteen from hips by GC-MS. The major compounds detected in the leaves were squalene (38.21%), ethane, 1,1-diethoxy- (9.65%), β-D-glucopyranose, 1,6-anhydro- (8.55%), furfural (5.50%) and 2-furancarboxaldehyde 5-(hydroxymethyl)- (5.19%). The major compounds in the hips were 2-furancarboxaldehyde 5-(hydroxymethyl)- (51.27%), β-D-glucopyranose, 1,6-anhydro- (8.18%), 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (7.42%), 2,5-furandione, dihydro-3-methylene- (6.79%) and furfural (5.99%). Current findings indicate that extract from leaves and hips of *Rosa abyssinica* and the bioactive components present could be used as pharmaceutical agents.

Keywords: *Rosa abyssinica,* antimicrobial, phytochemicals.

INTRODUCTION

Rosa abyssinica R. Br. ex Lindl. (Rosaceae) is a plant mostly found in the east Africa, Yemen and in southwest Saudi Arabia in Asir and Higaz regions (Browicz and Zielinski, 1991). Sori *et al*., (2004) described the plant as one of seventy-seven different plants have been used by Borana pastoralists to treat or prevent a wide range of livestock disease especially skin problems. The methanol extracts of *Rosa abyssinica* has the ability to lower carrageenan-induced paw oedema and the acetone fraction showed anti-inflammatory activity and inhibited formalin-induced nociception in mice (Sewuye and Asres, 2008). Thus, *Rosa abyssinica* Lindley is a potential medicinal plant that must be investigated to establish its antimicrobial activity. Since the extracts gained from some of *Rosa* spp. exhibited antibacterial activity against a variety of pathogenic microbes (Yilmaz and Ercisli, 2011; Nowak *et al*., 207; Yi *et al*., 2007; Khan and Tewari, 2011; Hirulkar and Agrawal, 2010). Also, members of the Rosaceae family are rich in bioactive compounds, as for example, Dog rose (*Rosa canina* L.) fruit has a high amount of ascorbic acid (Nojavan *et al*., 20008). Hips of *Rosa* spp. showed the presence of flavonoids, citric acid, ascorbic acid, citric acid and vitamin C (Artur *et al*., 2012). Phenolic compounds in Rose hips showed many physiological functions for example, ability to modify gene expression in microorganism, antimutagenic, antioxidant and anticargionogenic effects that gives the medicinal properties to the plant (Nakamura *et al*., 2003). Oil-bearing-rose (*Rosa damascena* Mill.) have been analyzed for its phytochemicals and found that it contains a varied amounts from phenol, flavanol, flavonols, Gallic acid, (+)-catechin and (-)-epicatechin compounds (Baydar and Baydar, 2013). Volatile compounds in flower of *rosa rugosa* are alcohols, ethers, esters and some alkanes, mainly including citronellol (40.38%), gerariol (13.49%), alcohol (12.29%), myrcene (7.12%), α-pinene (5.65%) whereas about 33 volatile compounds were identified (Feng *et al*., 2010). Previous studies have examined the bacteriostatic ability of these phytocomponents against some pathogenic microorganism found that they have a potent activity against a wide variety of microorganisms (Ceyhan *et al*., 2011; Gallucci *et al*., 2010; Togashi *et al*., 2007; Gonca *et al*., 2011; Bozdag-Dundar *et al*., 2003). Therefore, this study aimed to evaluate the antimicrobial potential of some solvent extracts from leaves and hips of *Rosa abyssinica* growing naturally in El-Soda, Abha region, Saudi Arabia against some gram (-) and gram (+) and two clinical isolates of *Candida* species. In reality, nothing is known about phytochemicals variability in leaves and hips of *Rosa abyssinica*, hence our study also aimed to determine if there is any chemical variability to differentiate between antimicrobial efficacies associated with them.

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MATERIALS AND METHODS

Experimental materials

A healthy fresh leaves and hips of *Rosa abyssinica* R. Br. ex Lindl. were collected from El Sauda mountains (2270 meters height), Abha region, Kingdom of Saudi Arabia. The voucher specimen stored in the herbarium of the Faculty of Science, King Khalid University.

Preparation of leaves and hips extracts

Ninty grams from *Rosa abyssinica* leaves and hips were washed thoroughly by distilled water and crushed directly by grinder (Thomas Wiley laboratory mill, model 4) for 15 minutes, and the solution samples were filtered through 2-layered muslin cloth (Girish and Satish, 2008). The filtrate was divided into seven aliquots each contains 20ml and subjected to fresh extraction by adding 20 ml from water, chloroform, petroleum ether, methanol diethyl ether, and acetone. Extraction from dry materials was done by adding 20ml from water, chloroform, petroleum ether, methanol diethyl ether, and acetone to the half-gram (0.5g) air-dried powdered leaves and hips materials. All sample were kept in rotary shaker at 99 rpm at 24°C for 48 hours. The resultant extract was filtered and placed in incubator at 39°C until the solvent was evaporated completely. Each extract was weighed, dissolved in sterile dimethyl sulfoxide (DMSO) and subjected to the antimicrobial activity test (Alamri and Moustafa, 2012).

Test organisms used

The test organisms used in this study were obtained from the Microbiology Laboratory, Faculty of Science and Faculty of Medicine, King Khalid University, KSA. The clinical isolates consisted of seven bacterial species; *S. aureus*, *K. pneumoniae*, *K. oxytoca*, *P. mirabilis*, *P. aeruginosa*, *M. luteus* and *Shigella* sp. and two pathogenic *Candida* spp.. All the test strains were first subcultured in nutrient broth at 37°C for 24 hours for bacterial strains and at 35ºC for 48 h for *Candida* spp. that used as test pathogens.

Screening for antimicrobial activity

Seven bacterial strains and two pathogenic *Candida* spp. were included in this study to investigate the efficacy of antimicrobial activities of various solvent extract gained from leaves and hips of *Rosa abyssinica*. We used well diffusion method to evaluate the antimicrobial activity according to (Patel *et al*., 2007; Alrumman *et al*., 2012). 20ml from sterilized Mueller Hinton agar (Oxoid, England) media was poured into sterile Petri dish for test bacteria and *Candida* spp. About 0.1ml of standardized inoculums of each test bacterium and *Candida* spp. was spread onto agar surface by using L shaped sterile glass spreader to get a uniform lawn culture. By using a sterile cork-borer, 6 mm diameter well was cut from the agar and each well was filled with 0.1ml of the solvent plant extraction. All Petri dishes were kept at 24°C temperature for one hour so the extract will diffuse properly into agar. Each extract was assayed in triplicate and sterile dimethyl sulfoxide (DMSO) served as negative and cefoxitin (30 mcg) as positive controls. Inoculated Petri dishes were kept at 30°C for 24 h for bacteria and 48 h for *Candida* spp. The antimicrobial activity was determined according to (Pundir and Jain, 2011).

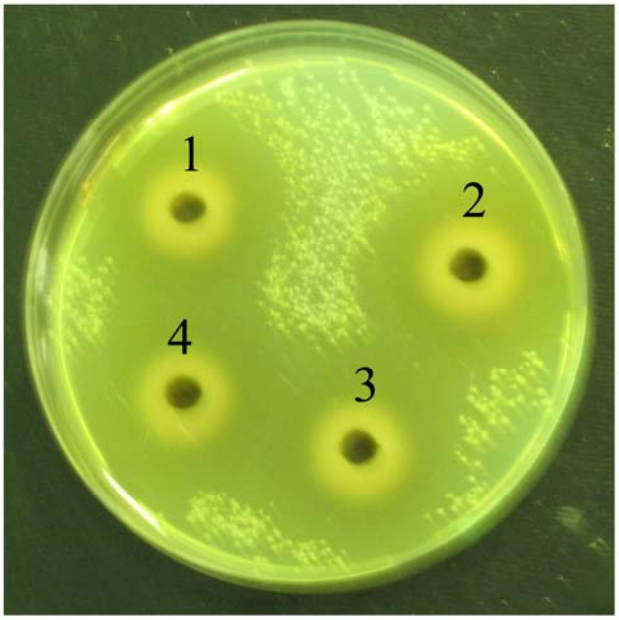

Fig. 1: *In vitro* effect of methanol (1), chloroform (2), petroleum ether (3), and acetone (4) leaves extract of *Rosa abyssinica* against pathogenic *Candida* sp.

STATISTICAL ANALYSIS

Analysis of variance was applied using SPSS to determine statistically significant differences amongst plant extracts. Data at *p*- value between 0.00 and 0.05 have been counted as statistically significant.

GC-MS analysis of leaves and hips

The analyses of the leaves and hips extracts were done using a Clarus 500 Perkin - elmer (Auto system XL) Gas Chromatograph having an Elite -1 (100% Dimethyl polysiloxane) and TR-V1 column. Helium gas (99.999%) was applied as a carrier gas at conditions of flow rate of (6.0 mL/min) and volume injection (2µL), split ratio (5: 1) and injector heat 200°C. The oven heat was programmed to temperature of 35°C -4 minutes hold then 30°C/minute to 90°C then 30°C/minute to 110°C -No hold, then 45°C/minute to 170°C hold for 1 minute. Spectra were recorded at 70 eV; an interval scan of 0.5 s and at the fragments between 40 to 550 Da (Ezhilan and Neelamegam, 2012; Moustafa *et al*., 2013). Identification of mass spectrum GC-MS was interpreted using information of National Institute Standard and Technology (NIST) having more than sixty-two thousand of patterns. Unknown chemicals present in the leaves and

hips of *Rosa abyssinica* were identified by matching spectrum of known components stored in the National Institute Standard and Technology library.

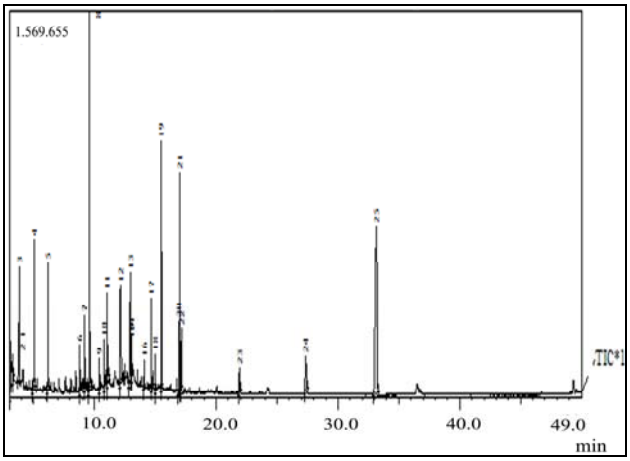

Fig. 2: A typical GC-MS chromatogram of the chemical constituents of ethanol extract of *Rosa abyssinica* leaves.

RESULTS

Susceptibility of the microbes to different solvent extracts

The results of the antimicrobial activities using well diffusion method gained from water, chloroform, petroleum ether, methanol diethyl ether, and acetone extracts of fresh and dried leaves and hips of *Rosa abyssinica* are presented in table 1. An analysis (one way ANOVA) of the results clearly indicated that there are a statistically significant effect of *Rosa abyssinica* on antimicrobial activities owing to the plant parts used and the solvent type. The cold-water extract of dry leaves and hips showed no zones of inhibition against any microbes tested. The hot water extracts from dry and fresh leaves inhibited 6 out of the 9 (66.66%) microbes used with average zone diameters ranging from 8.50±14.72 against *Candida* sp. to 20.13±0.40 against *M. luteus*. The cold water extracts from dry and fresh hips did not show any inhibition against all tested pathogens. Methanol, chloroform, petroleum ether, acetone and diethyl ether extracts gained from dry and fresh leaves and methanol, chloroform, petroleum ether extract gained from dry and fresh hips inhibited all tested microorganism (100%). Diethyl ether extract of dry and fresh hips inhibited 6 out of the 9 (66.66%) microbes and acetone inhibited 4 out of the 9 (44.44%). Chloroform, petroleum ether and methanol extract from the fresh leaves showed strong antimicrobial activity against *Candida* species (fig. 1) with zones of inhibition ranging from 37.06±0.75 to 23.86±0.66 mm. Methanol and acetone extract from the fresh leaves demonstrated strong activity against *P. mirabilis* with zones of inhibition ranging from 28.73±0.40 to 28.46±0.49mm, while the extract from the dry leaves having antimicrobial activity with zones of inhibition ranging from 25.80±0.36 to 25.60±0.435mm. Chloroform and petroleum ether showed good antibacterial activities against *K. pneumonia* and *K. oxytoca* with zones of inhibition ranging from 29.46±0.25 to 21.83±0.61 mm. Acetone and dethyl ether extracts gained from fresh hips showed modest activity against *K. pneumonia* (17.70±0.100), *P. mirabilis* (17.50±0.80) respectively compared to the respective dry extracts. Methanol, chloroform and petroleum ether showed better activity against *Candida* sp. ranged from (20.36±0.15 to 20.03±0.51) compared to the respective dry extracts. Dimethyl sulfoxide (DMSO) which have been used as a negative control in the wells did not show any effect on microbial growth, while the positive control (Cefoxitin-30 mcg) produced a clear zone in all tested plates (table 1).

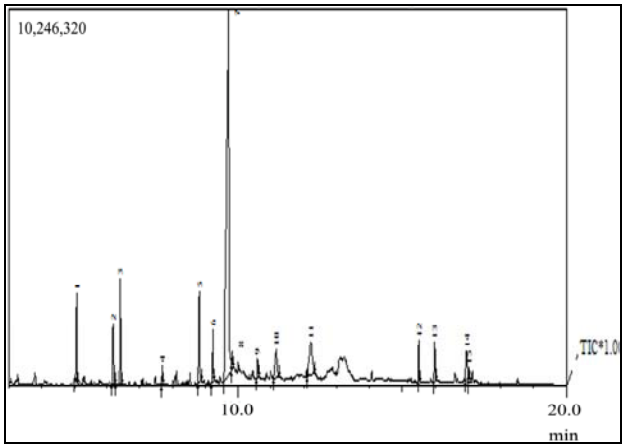

Fig. 3: A typical GC-MS chromatogram of the chemical constituents of ethanol extract of *Rosa abyssinica* hips.

Chemicals in the leaves and hips

GC-MS chromatogram analysis of the ethanol extract of leaves and hips of *Rosa abyssinica* showed twenty-five peaks for leaves and fifteen peaks for hips (figs. 2, 3). Identified and characterized phytochemicals present in the leaves and hips extracts, with their chemical formula, retention period, molecular weight, and peak area percents are presented in (table 2 and 3). Of the twenty-five compounds identified in the leaves, the most prevailing compounds were squalene (38.21%), ethane, 1,1-diethoxy- (9.65%), β-D-glucopyranose, 1,6-anhydro- (8.55%), furfural (5.50%) and 2-furancarboxaldehyde 5-(hydroxymethyl)- (5.19%). 4h-pyran-4-one, 2,3-dihydro-3, 5-dihydroxy-6-methyl-, 3-heptanol, 2,6-pyridinedicarboxylic acid, azelaic acid, n-hexadecanoic acid, 1-octadecyne, phytol and octadecanoic acid found to be the minor components of leaves sample (<1%). Of the fifteen compounds identified in the hips, the most dominant chemicals were 2-furancarboxaldehyde 5-(hydroxymethyl)- (51.27%), β-D-glucopyranose, 1,6-anhydro- (8.18%), 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (7.42%), 2,5-furandione, dihydro-3-methylene- (6.79%) and furfural (5.99%). N-hexadecanoic acid and 9,12,15-octadecatrienoic acid, (Z,Z,Z)- were identified to be minor components of the hips sample.

Table 1: Susceptibility of microbes to the extract of leaves and hips from *Rosa abyssinica*

Pathogen	Solvent	Mean diameter of zone of inhibition (mm) ± SD at concentration of 0.33 g/ml (95% Confidence interval for mean; lower - upper bound)					
		Dry leaves	Fresh leaves	Dry hips	Fresh hips	(Cefoxitin-30 mcg)	DMSO
S. aureus	Methanol	16.66±0.49** (15.44-17.8)	19.90±0.608** (18.38-21.41)	16.66±0.49** (15.44-17.89)	19.06±0.49** (17.84-20.29)	24.76± 1.778 (25.58-31.61)	NIZ
	Chloroform	14.87±1.374** (11.45-18.28)	17.96±1.66** (13.83-22.09)	9.26±0.64** (7.66-10.86)	10.80±0.51** (9.50-12.09)	" " "	NIZ
	Petroleum ether	13.76±0.305** (13.00-14.52)	16.56±0.404** (15.56-15.56)	13.53±0.25** (12.90-14.15)	15.6±0.17** (15.16-16.03)	" " "	NIZ
	Acetone	9.933±0.152** (9.55-10.31)	11.90±0.458** (10.76-13.03)	NIZ	NIZ	" " "	NIZ
	Diethyl ether	22.23±0.152* (20.48-23.97)	26.53±0.802* (24.54-28.52)	NIZ	NIZ	" " "	NIZ
	Hot water	9.43±0.416** (8.39-10.46)	11.23±0.513** (9.95-12.50)	NIZ	NIZ	" " "	NIZ
	Cold water	NIZ	NIZ	NIZ	NIZ	" " "	NIZ
Shigella sp.	Methanol	19.96±0.750** (18.09-21.82)	20.76±0.611* (19.24-22.28)	9.40±0.60** (7.88-10.91)	12.5±0.72** (10.70-14.29)	28.6±1.212 (25.58-31.61)	NIZ
	Chloroform	17.50±0.435** (16.41-18.58)	24.03±6.76* (7.22-40.84)	8.20±0.20** (7.703-8.69)	10.80±0.30** (10.05-11.54)	" " "	NIZ
	Petroleum ether	26.8±0.556* (25.41-28.18)	30.0±0.65 (28.37-31.62)	8.30±0.10** (8.05-8.54)	10.93±0.15** (10.55-11.31)	" " "	NIZ
	Acetone	20.43±0.577** (18.99-21.86)	22.86±0.63* (21.28-24.44)	NIZ	NIZ	" " "	NIZ
	Diethyl ether	20.10±0.200** (19.60-20.59)	22.50±0.20* (22.00-22.99)	9.10±0.10** (8.85-9.34)	11.23±0.25** (10.60-11.85)	" " "	NIZ
	Hot water	10.30±0.300** (9.55-11.04)	11.50±0.30 (10.75-12.24)	NIZ	NIZ	" " "	NIZ
	Cold water	NIZ	NIZ	NIZ	NIZ	" " "	NIZ
M. luteus	Methanol	25.70±0.400* (24.70-26.69)	28.30±0.400** (27.30-29.29)	9.73±0.25** (9.10-10.35)	12.46±0.30** (11.70-13.22)	24.73±0.47 (23.55-25.90)	NIZ
	Chloroform	20.53±0.400** (19.52-21.53)	22.66±0.55** (21.29-24.03)	8.30±30.30** (7.57-9.092)	10.70±0.50** (9.45-11.94)	" " "	NIZ
	Petroleum ether	22.33±0.660** (20.67-23.98)	24.56±0.72* (22.76-26.36)	18.13±0.40** (17.12-19.13)	19.66±5.60* (5.75-33.57)	" " "	NIZ
	Acetone	23.83±0.400* (22.82-24.83)	26.23±0.40* (25.22-27.23)	NIZ	NIZ	" " "	NIZ
	Diethyl ether	20.76±0.760** (18.86-22.66)	22.86±0.86* (20.72-25.00)	13.53±0.40** (12.52-14.53)	15.23±1.00** (12.74-17.72)	" " "	NIZ
	Hot water	18.23±0.350** (17.36-19.10)	20.13±0.40** (19.12-21.13)	NIZ	NIZ	" " "	NIZ
	Cold water	NIZ	NIZ	NIZ	NIZ	" " "	NIZ
P. aeruginosa	Methanol	15.39±0.462** (14.24-16.54)	18.46±0.66** (16.81-20.12)	9.63±0.47** (8.45-10.80)	12.60±0.62* (11.04-14.15)	21.40±0.620 (19.84-22.95)	NIZ
	Chloroform	19.86±0.251** (19.24-20.49)	23.80±0.40** (22.80-24.79)	8.13±0.208** (7.616-8.65)	10.60±0.26* (9.94-11.25)	" " "	NIZ
	Petroleum ether	15.733±0.37** (14.79-16.67)	18.86±0.49** (17.64-20.09	13.6±0.264** (12.94-14.25)	16.00±2.68* (9.32-22.67)	" " "	NIZ
	Acetone	23.86±0.25** (23.24-24.49)	28.50±0.30** (27.75-29.24)	NIZ	NIZ	" " "	NIZ
	Diethyl ether	21.7±0.529 (20.38-23.01)	25.90±0.62** (24.34-27.45)	9.50±0.264** (8.842-10.15)	13.23±0.32* (12.43-14.03)	" " "	NIZ
	Hot water	14.40±0.43** (14.24-16.54)	17.16±0.55** (15.79-18.53)	NIZ	9.60±8.32** (-11.09-30.29)	" " "	NIZ
	Cold water	NIZ	NIZ	NIZ	NIZ	" " "	NIZ

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K. pneumoniae	Methanol	20.53±0.351** (19.66-21.40)	23.63±0.51 (22.35-24.90)	8.40±0.264** (7.742-9.057)	10.93±0.37** (9.99-11.87)	23.30±1.050 (20.67-25.92)	NIZ
	Chloroform	20.4±0.360** (19.50-21.29)	23.43±0.32 (22.63-24.23)	8.36±0.305** (7.607-9.125)	10.86±0.40** (9.86-11.87)	" " "	NIZ
	Petroleum ether	25.63±0.305** (24.87-26.39)	29.46±0.25** (28.84-30.09)	8.53±0.378** (7.592-9.473)	11.133±0.56** (9.72-12.54)	" " "	NIZ
	Acetone	20.57±0.410** (19.54-21.59)	23.76±0.500 (22.51-25.01)	13.60±0.100** (13.35-13.84)	17.70±0.100** (17.45-17.94)	" " "	NIZ
	Diethyl ether	19.53±0.321** (18.73-20.33)	22.36±0.37* (21.42-23.30)	9.53±0.378** (8.592-10.47)	12.1±0.95** (9.73-14.46)	" " "	NIZ
	Hot water	10.73±0.476** (19.66-21.40)	12.30±0.520** (10.98-13.61)	9.90±0.721** (8.108-11.69)	12.86±0.97** (10.45-15.27)	" " "	NIZ
	Cold water	NIZ	NIZ	NIZ	NIZ	" " "	NIZ
K. oxytoca	Methanol	21.6±0.300** (20.85-22.34)	24.6±0.30** (23.85-25.34)	9.63±0.378** (8.692-10.57)	15.66±7.47** (-2.90-34.24)	33.03±5.601 (19.11-46.94)	NIZ
	Chloroform	25.76±0.251** (25.14-26.39)	29.40±0.20* (28.90-29.89)	8.43±0.152** (8.053-8.81)	9.66±0.200** (9.14-10.18)	" " "	NIZ
	Petroleum ether	19.20±0.55** (17.81-20.58)	21.83±0.61** (20.31-23.35)	9.40±0.360** (8.504-10.29)	10.80±0.450** (9.66-11.93)	" " "	NIZ
	Acetone	25.36±0.723** (23.56-27.16)	28.90±0.78* (26.95-30.84)	9.63±0.288** (8.916-10.35)	11.10±0.340** (10.23-11.96)	" " "	NIZ
	Diethyl ether	25.39±0.513** (24.12-26.67)	28.93±0.57* (27.49-30.36)	10.0±0.818** (7.966-12.03)	11.50±0.910** (9.22-13.77)	" " "	NIZ
	Hot water	NIZ	NIZ	8.73±0.568** (7.320-10.14)	NIZ	" " "	NIZ
	Cold water	NIZ	NIZ	NIZ	NIZ	NIZ	NIZ
P. mirabilis	Methanol	25.80±0.36** (24.90-26.69)	28.73±0.40** (27.72-29.73)	NIZ	NIZ	23.13±0.850 (21.02-25.24)	NIZ
	Chloroform	25.6±0.346** (24.73-26.46)	28.50±0.34** (27.63-29.36)	NIZ	NIZ	" " "	NIZ
	Petroleum ether	23.46±0.40 (22.46-24.47)	26.16±0.45** (25.04-27.28)	NIZ	NIZ	" " "	NIZ
	Acetone	25.60±0.435** (24.51-26.68)	28.46±0.49** (27.24-29.69)	15.43±0.05** (15.28-15.57)	17.20±0.00** (17.20-17.2)	" " "	NIZ
	Diethyl ether	21.50±0.400** (20.50-22.49)	23.93±0.45 (22.81-25.05)	15.73±0.60** (14.23-17.23)	17.50±0.80** (15.51-19.48)	" " "	NIZ
	Hot water	10.33±0.25** (9.708-10.95)	11.46±0.30** (10.70-12.22)	11.83±0.60** (10.33-13.33)	13.1±0.65** (11.47-14.72)	" " "	NIZ
	Cold water	NIZ	NIZ	NIZ	NIZ	" " "	NIZ
C. albicans	Methanol	20.73±0.56** (19.32-22.14)	23.86±0.66** (22.21-25.52)	10.63±0.65** (9.01-9.01)	12.83±0.75** (10.96-14.69)	18.56±0.55 (17.19-19.93)	NIZ
	Chloroform	23.8±0.26 ** (23.14-24.45)	27.30±0.26** (26.64-27.95)	13.63±0.30** (12.87-14.39)	16.50±0.30** (15.75-17.24)	" " "	NIZ
	Petroleum ether	25.53±0.20 ** (25.016-26.05)	29.36±0.15** (28.98-29.74)	16.83±0.20** (16.31-17.35)	20.36±0.15** (19.98-20.74)	" " "	NIZ
	Acetone	20.38±0.45 ** (19.26-21.49)	23.50±0.69** (21.77-25.22)	16.6±0.45** (15.46-17.73)	20.03±0.51** (18.75-21.30)	" " "	NIZ
	Diethyl ether	25.30±0.340** (24.43-26.16)	29.03±0.40** (28.029-30.03)	NIZ	NIZ	" " "	NIZ
	Hot water	NIZ	NIZ	NIZ	NIZ	" " "	NIZ
	Cold water	NIZ	NIZ	NIZ	NIZ	" " "	NIZ
Candida sp.	Methanol	27.16±6.140 (11.91-42.42)	31.2±7.03* (13.72-48.67)	13.73±0.47** (12.55-14.90)	15.70±0.52** (0.30-14.38)	18.90±0.51 (17.60-20.19)	NIZ
	Chloroform	32.26±0.650* (30.65-33.88)	37.06±0.75* (35.20-38.93)	14.73±0.20** (14.21-15.25)	16.86±0.35** (0.20-15.99)	" " "	NIZ
	Petroleum ether	29.76±2.800* (22.80-36.72)	34.16±3.2*3 (26.13-42.20)	17.43±0.41** (16.39-18.46)	19.86±0.47* (0.27-18.69)	" " "	NIZ
	Acetone	25.56±2.62 (19.03-32.09)	29.3±3.03* (21.76-36.83)	9.76±0.45** (8.64-10.88)	11.13±0.50** (0.29-9.88)	" " "	NIZ
	Diethyl ether	25.73±0.208 (25.21-26.25)	29.53±0.20* (29.01-30.05)	9.66±0.47** (8.49-10.84)	11.0±0.52** (0.30-9.68)	" " "	NIZ
	Hot water	8.50±14.72* (-28.07-45.07)	9.76±16.91 (-32.25-51.78)	NIZ	NIZ	" " "	NIZ
	Cold water	NIZ	NIZ	NIZ	NIZ	" " "	NIZ

NIZ = No inhibition zone; DMSO, dimethyl sulfoxide (negative control); Cefoxitin-30 mcg, (positive control). * $p<0.05$, ** $p<0.01$, represent

Table 2: GC-MS analysis of ethanol extract of *Rosa abyssinica* leaves

No.	Compounds	Rt Time	% Area	M.W.	Chemical Formula
1	Ethoxyacetic acid	3.235	2.951401	104.10	$C_4H_8O_3$
2	Methane, diethoxy-	3.306	1.803323	104.14	$C_5H_{12}O_2$
3	Ethane, 1,1-diethoxy-	3.880	9.653645	118.17	$C_6H_{14}O_2$
4	Furfural	5.072	5.496653	96.08	$C_5H_4O_2$
5	Ethanol, 2,2-diethoxy-	6.178	3.198317	134.17	$C_6H_{14}O_3$
6	4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	8.794	0.910343	144.12	$C_6H_8O_4$
7	Pentanoic acid, 4-oxo-	9.209	1.65159	116.11	$C_5H_8O_3$
8	2-furancarboxaldehyde 5-(hydroxymethyl)-	9.591	5.190711	126.11	$C_6H_6O_3$
9	3-Heptanol	10.405	0.226991	116.20	$C_7H_{16}O$
10	Methyl 2-oxopentanoate	10.787	1.984244	130.14	$C_6H_{10}O_3$
11	1,2,3-benzenetriol	11.043	3.999822	126.11	$C_6H_6O_3$
12	β-D-glucopyranose, 1,6-anhydro-	12.127	8.54545	162.14	$C_6H_{10}O_5$
13	α-D-glucopyranoside, methyl	-	-	194.18	$C_7H_{14}O_6$
14	2,6-pyridinedicarboxylic acid	13.019	0.595087	167.11	$C_7H_5NO_4$
15	Azelaic acid	13.078	0.4375	188.22	$C_9H_{16}O_4$
16	n-hexadecanoic acid	14.074	0.26902	256.42	$C_{16}H_{32}O_2$
17	1-octadecyne	14.667	0.762237	250.46	$C_{18}H_{34}$
18	Phytol	14.977	0.237569	296.54	$C_{20}H_{40}O$
19	n-hexadecanoic acid	15.504	2.859094	256.42	$C_{16}H_{32}O_2$
20	1,E-11,Z-13-octadecatriene	16.938	1.382704	248.44	$C_{18}H_{32}$
21	9,12,15-octadecatrienoic acid, (Z,Z,Z)-	17.026	3.261801	278.42	$C_{18}H_{30}O_2$
22	Octadecanoic acid	17.140	0.928189	284.47	$C_{18}H_{36}O_2$
23	Tridecane	21.915	1.544864	184.36	$C_{13}H_{28}$
24	Eicosane	27.362	3.902526	282.54	$C_{20}H_{42}$
25	Squalene	33.163	38.20692	410.71	$C_{30}H_{50}$

Table 3: GC-MS analysis of ethanol extract of *Rosa abyssinica* hips

No.	Compounds	Rt Time	% Area	M.W.	Chemical Formula
1	Furfural	5.075	5.986003	96.08	$C_5H_4O_2$
2	Ethanol, 2,2-diethoxy-	6.189	2.935343	134.17	$C_6H_{14}O_3$
3	2,5-furandione, dihydro-3-methylene-	6.414	6.791494	112.08	$C_5H_4O_3$
4	Pentanoic acid, 4-oxo-	7.683	1.581273	116.11	$C_5H_8O_3$
5	4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	8.814	7.419011	144.12	$C_6H_8O_4$
6	Propanoic acid,2-(acetyloxy)-2-methyl-, ethyl ester	9.238	4.329486	174.19	$C_8H_{14}O_4$
7	2-furancarboxaldehyde 5-(hydroxymethyl)-	9.713	51.27459	126.11	$C_6H_6O_3$
8	1,2,3-propanetriol, monoacetate	9.817	2.764988	134.13	$C_5H_{10}O_4$
9	1-pentanol	10.598	1.6072	88.15	$C_5H_{12}O$
10	2-furanmethanol, tetrahydro-	11.166	2.969602	102.13	$C_5H_{10}O_2$
11	β-D-glucopyranose, 1,6-anhydro-	12.225	8.182214	162.14	$C_6H_{10}O_5$
12	n-hexadecanoic acid	15.507	0.834308	256.42	$C_{16}H_{32}O_2$
13	2-cyclohexene-1-carboxylic acid, 1-methyl-4-oxo-, ethyl ester	15.990	1.590905	182.22	$C_{10}H_{14}O_3$
14	Oleic acid	16.972	1.347226	282.46	$C_{18}H_{34}O_2$
15	9,12,15-octadecatrienoic acid, (Z,Z,Z)-	17.092	0.38636	278.42	$C_{18}H_{30}O_2$

DISCUSSION

There is believe among leading scientists to find alternative candidates against different diseases using herbal medicine as they considered non-toxic. Since, we did not have safe modern synthetic drugs 100%, so World Health Organization (WHO) has recommended the evaluation of the effectiveness of plants as an alternatives drugs (Mohseni-Salehi-Monfared *et al*., 2010). Hence, the recent years witnessed attention many scientists to do a research on plant kingdom as a main source to control human illness and to discover biological active compounds (Woldemichael *et al*., 2003; Nasar-Abbas and Halkman, 2004; Yi O *et al.,* 2007; Pulit and Banach, 2014). In this regard, antimicrobial affects of extract from leaves and hips of *Rosa abyssinica* R. Br. ex Lindl.

(Rosaceae) on pathogenic microbes have not been documented. Based on the result, it was concluded that the extract of *Rosa abyssinica* possesses significant antimicrobial activity. Extraction by some solvents showed a potent antimicrobial activity when compared with standard compound. Our results could be significant since it was reported that there is a high prevalence of clinical pathogens to resist to antibiotic such as chloramphenicol, streptomycin, ampicillin and sulphonamides (US Food and Drug Administration 2008; Kozak *et al*., 2009; Daniel *et al*., 2012). The methanol, chloroform, petroleum ether, acetone and diethyl ether extracts have a greater effect on pathogenic *Candida* sp. than that of hot water extraction. These results, in agreement with previous findings indicate that the most of the antimicrobial active compounds were soluble in polar and non-polar solvents such as methanol, acetone, chloroform, petroleum ether and diethyl ether instead of water (Ulukanli *et al*., 2011; Fred-Jaiyesimi *et al*., 2010; Najib *et al*., 2012; Parekh *et al*., 2006). It was noted that the type of solvent and extraction conditions affected the degree of antibacterial activity (Wendakoon *et al*., 2012). The maximum antibacterial properties gained from fresh leaves petroleum ether extract was found against *Shigella* sp., and *K. pneumonia* in the form of zone of inhibition. Moreover, acetone, diethyl ether, methanol and chloroform extracts of fresh leaves strongly inhibited the growth of *P. aeruginosa*, *M. luteus* and *K. oxytoca* more strongly than corresponding extract from dried leaves, a justification that the inhibition was not affected by the type of solvent used but also extraction condition from plant materials (fresh or dry). In general, extract from fresh leaf or hips were more effective than those that had been dried in the oven or sunshine indicating that the active principles are subjected to degradation. A previous study indicated that the antibacterial activity was obviously decreased after drying *Ocimum sanctum* (Mondal *et al*., 2007), *Zingiber officinale* (Sasidharan and Menon, 2010) and *Lippia gracilis (*Bitu *et al*., 2012). Also, using convective drying will cause a significant loss in volatile compound and the loss will increase with increasing the temperature (Díaz-Maroto *et al*., 2004). It is evident to keep most of the plant components as it especially for volatile oil, which is somewhat guaranteed in the fresh sample (Al-Jaber *et al*., 2012). Although, the-freeze drying is one of the best methods in pharmaceuticals techniques for the medicinal plants but it cause a relatives changes in the molecules concentration that influence the plant pharmaceutical properties (Abascal *et al*., 2005). For these reasons and our finding, we can conclude that drying may have a harm effect on plant active principles than to perform fresh extraction. The results of this study also showed that the antimicrobial activity is highly correlated with the type of the plant parts investigated. The inhibition zone registered from *Rosa* abyssinica leaves dry or fresh against a number of pathogenic bacteria and *Candida* sp. higher than that corresponding *Rosa abyssinica* hips. The least inhibition zone were obtained from fresh and dry hips chloroform extract against *S. aureus*, *Shigella* sp., *P. aeruginosa*, *M. luteus K. pneumonia* and *K. oxytoca*. Methanol and diethyl ether extract gained from fresh hips *Rosa* abyssinica showed very low activity against *Candida* spp. Previous studies indicated that the hip extract from *R. nutkana* and *R. pisocarpa* from British Columbia has antimicrobial activity on yeast growth and Gram-positive bacteria (Yi *et al*., 2007). The variation in antimicrobial activities between leaves and hips may be due to the differences in accumulation of plant phytochemicals in particular parts or correlated with plant phenological stage (Németh, 2005; Nejad *et al*., 2008). The antimicrobial activity found in the hips of *Rosa* abyssinica could explain that some rose hips are used to prepare 'Nypon soppa', the traditional Swedish fruit soup, as herbal tea in Europe and as marmalade and fruit juice in the Tokat region of Anatolia, Turkey (Gune *et al*., 2010). Historically, products from rose hips are widely consumed in many regions of Portugal (Barros *et al*., 2011) and recommended for their utilization as functional foods and as edible colorants in Romania (Rosu *et al*., 2011), so Saudi Arabian weed *Rosa abyssinica* needs effective study for its particular nutritional uses.

The results pertaining to GC-MS analysis showed the existence of thirty-nine different phytochemicals in leaves and hips and almost of the identified compounds showed biological activities. For example, furfural and its derivatives (Sutar *et al*., 2012), α-D -glucopyranoside and eleven of its decanoylation derivatives were reported as having antimicrobial activities (Kabir *et al*., 2005). Pyridine-2,6-dicarboxylic acid complexes have many uses in the area of antitumor activity, processing of more promising anti-HIV drugs, antibacterial agents, developments of insulin-mimetic chemicals and many other activities (Kirillova *et al*., 2007; Vargova *et al*., 2004; Park *et al*., 2007; Moghimi *et al*., 2007). Identified twenty-four bioactive components in the leaves, explained its higher antimicrobial activities than the hips, which have only fifteen compounds. These characterized chemicals might be used as the reference compounds in determining the efficacy the antimicrobial activities for the plant.

CONCLUSION

It is evident that leaves and hips of *Rosa abyssinica* have antimicrobial activity, deserving further study for drugs applications. The bioactive compounds found can be utilized for the development of natural antibiotic against pathogenic microbes.

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