Phytochemical Screening and Assessment of Antioxidant and Antimicrobial Activities of the Root extracts of Three Sudanese Citrus Species

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Abstract

Citrus fruits, which belong to the family Rutaceae, biosynthesize and accumulate in their cells a great variety of phytochemicals including alkaloids, terpenoids, flavonoids, saponins, coumarins, tannins and others. In the present investigation the roots of *Citrus paradisi, C.aurantium*, and *C.sinensis* have been selected and subjected to phytochemical screening followed by assessment of their antimicrobial and antioxidant activities.

Observations on the antibacterial activity showed that *Staphylococcus aureus* was the most sensitive (30 mm inhibition zone) followed by *Bacillus subtilis* (25 mm inhibition zone) against *C. Paradisi* methanolic extract. The chloroform extracts of the three species were active against *C. Albicans* (15-20 mm inhibition zone). The methanolic extracts of *C.sinensis* root was devoid of any antifungal activity.

The root methanolic extract of *C.sinensis* showed the highest antioxidant activity (80 ± 0.02) amongst the tested extracts of the three citrus species.

Keywords: *Citrus paradisi; Citrus sinensis; Citrus aurantium*; Rutaceae; Phytochemical Screening; Activity; Antibacterial; Antioxidant.

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Introduction

Citrus fruits, which belong to the family of Rutaceae, are one of the main fruit tree crops grown throughout the world. Although *Citrus sinensis* (sweet orange) is the major fruit in this group accounting for about 70% of citrus output, the group also encompasses small citrus fruits such as *Citrus paradisi* (grape fruit), *Citrus aurantium, Citrus reticulata* (tangerine tree), *Citrus aurantifolia* (lime tree) and *Citrus limonum* (lemon tree) [1]. Citrus fruits are notable for their fragrance, partly due to flavonoids and limonoids (which in turn are terpenes) contained in the rind, and most are juice laden. The juice exhibit higher antimicrobial activity and contains a high quantity of citric acid giving them their characteristic sharp flavour. They are also good sources of vitamin C, flavanones and flavones [2-6]. Data about the chemical composition and uses of citrus plant roots are scant which prompted us to investigate root samples of three citrus species growing in Sudan.

The present work reports our results on phytochemical screening, antimicrobial and antioxidant activities of chloroform and methanol root extracts of *C. Paradisi; C. Sinensis and C. Aurantium.*

Material and Method

Collection and processing of plant samples

Roots of *Citrus paradisi, C. sinensis* and *C.aurantium,* were obtained from University of Khartoum Agricultural Faculty. The roots were taken in different trays and kept for drying under shade at room temperature for three weeks. The dried plant samples (roots) were taken separately and ground to obtain a coarse powder. The powdered samples were stored in a clean glassware container until needed for analysis.

Extraction

The extraction of roots of *C.paradisi, C. sinensis*, and *C.aurantium* was carried out using Soxhlet extraction procedure. The powdered samples of the three plants (800.0 g) of each were extracted sequentially with petroleum ether (60-80°C), chloroform and methanol for 72 hours. The extracts were filtered using Whatman filter paper (No.1) while hot, concentrated under reduced pressure using rotary evaporator, and dried in a desiccator. The extracts were then kept in sterile bottles, under refrigerated conditions, until further use.

Phytochemical Screening

The root chloroform and methanol extracts of the three samples were subjected to preliminary phytochemical testing to detect the presence of different chemical groups of compounds; such as saponins, tannins, alkaloids, flavonoids, triterpenoids, steroids, glycosides, anthraquinones, coumarins, carbohydrates, and reducing sugars, as described in the literature [7-9].

Microorganisms

The test microorganisms *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* NCTC 8236, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC7596) were sub cultured onto nutrient agar in order to determine their viability. The identity of each test organism was confirmed using standard cultural, morphological and biochemical techniques. Stock cultures were maintained on nutrient agar slants at 4°C and sub cultured in nutrient broth at 37°C prior to each antimicrobial test.

Assessment of antimicrobial activity

Antibacterial and antifungal activities of root extracts against four pathogenic bacteria (two Gram-positive and two Gram-negative) and one pathogenic fungi were assessed by the agar

disk diffusion method. The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines [10]. Bacterial suspension was diluted with sterile physiological solution to 10^8cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the Mueller-Hinton Agar (MHA) and soaked with 20 µl of a solution of each plants roots extracts which were made by using dimethyl sulphoxide (DMSO) as a diluting solvent. The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured and recorded. Ampicillin; Cephalexin and Amoxicillin were used as standard drugs to assess the antimicrobial activity.

Antioxidant Assay

The DPPH radical scavenging was determined according to method with some modification [11]. In 96-wells plate, the test samples were allowed to react with 2,2-Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37ºC. The concentration of DPPH was kept as (300µM). The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517nm using multiplate reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group. All tests and analysis were run in triplicate.

Results and Discussion

The phytochemical analysis of the pet ether, chloroform and methanol root extracts of *Citrus paradisi*, *C. sinensis* and *C. aurantium* were profiled for their secondary metabolites and results were presented in Table 1. The results confirmed the presence of alkaloids, tannins,

sterols, flavonoids, saponins, coumarins, reducing sugar, and carbohydrates in the methanolic and chloroform extracts with few exceptions. Anthraquinone and cyanogenic glycoside were not detected.

Results of phytochemical screening were reported and confirmed by direct detection from powdered roots of the three samples, following the same procedures.

Table 1: Results of phytochemical screening of *Citrus paradisi*, *C. sinensis* and *C. aurantium* root extracts

Phytochemical constituents	*Citrus paradisi* Root			*Citrus sinensis* Root			*Citrus aurantium* Root		
	RP	Meth extract	Chl extract	RP	Meth extract	Chl extract	RP	Meth extract	Chl extract
Alkaloids	+	+	±	+	±	+	+	+	+
Sterols	+	+	-	+	+	-	+	+	-
Triterpenes	+	-	+	+	-	+	+	-	+
Flavonoids	+	+	+	+	+	+	+	+	±
Saponins	+	+	-	+	±	-	+	+	-
Coumarins	+	±	+	+	+	-	+	+	+
Tannins	+	+	-	+	+	-	+	+	±
Anthraquinone glycoside	-	-	-	-	-	-	-	-	-
Cyanogenic glycoside	-	-	-	-	-	-	-	-	-
Carbohydrates	+	+	+	+	+	+	+	+	+
Reducing compounds	+	+	+	±	+	+	+	+	±

RP = Root powder, Meth = Methanol, Chl = Chloroform
(+) Positive, (-) Negative, (±) Traces

The result of antimicrobial activities of the root methanolic and chloroform extracts of the three samples were discussed according to their general phytochemical screening findings. The fungus *C. albicans* was moderately susceptible to methanolic and chloroform extracts of the three species (15-20 mm, IZD), but was resistant to the root methanolic extract of *C. sinensis*. Triterpenes were not present although they were detected in the crude sample of the roots. The Gram (+) ve bacteria *Staphylococcus aureus* (Sa) and *Bacillus subtilis* (Bs) were highly sensitive to the chloroform and methanol root extracts of the three plants (Sa: 10-30 mm IZD and Bs: 15-26 mm IZD). The Gram (-) ve bacteria *E. coli* (Ec) and *Pseudomonas aeruginosa* (Ps) were less sensitive to the methanolic and chloroform root extracts of the samples (Ec: 08-18mm IZD and Ps: 12-17 mm IZD) (Table 2).

Ampicillin, Cephalexin and Amoxicillin were used as standard antibiotic drugs. The Gram (-) ve Ec was resistant to Cephalexin, but the rest were sensitive to the three antibiotics in the range (15-25 mm, IZD) (Table 3).

Results of antioxidant activity of pet. ether, chloroform and methanol root extracts of *Citrus paradisi*, *C. sinensis* and *C. Aurantium* were reported in table (4). The methanolic root extract of *C. sinensis* showed the highest activity (80 ± 0.02), followed by methanol root extract of *C.paradisi* (52 ± 0,03) and chloroform root extract of *C. sinensis* (52 ± 0,02).

Conclusion

In conclusion, the root extracts of *C. sinensis* exhibited the highest antibacterial and antioxidant activities in the three species [12, 13], hence a starting point for more detailed investigations to determine their bioactive constituents.

Table 2: Results of assessment of Antimicrobial activities of methanol and chloroform root extracts of *Citrus paradisi, C. sinensis and C. aurantium*

Tested microorganism / Root Extracts	Antimicrobial Activity Inhibition Zone Diameter (IZD) in (mm) MIC= 100µg/ml				
	Ec	**Ps**	**Sa**	**Bs**	**Ca**
1- ***Citrus paradisi*** chloroform extract	15	12	16	26	20
methanol extract	08	17	30	25	17
2- ***Citrus sinensis*** chloroform extract	16	15	21	25	17
methanol extract	15	15	29	16	-
3- ***Citrus sinensis*** chloroform extract	18	17	18	25	15
methanol extract	13	12	10	15	15

Microorganisms:
Ec: *Escherichia coli*; **Ps**: *Pseudomonas aeruginosa*; **Sa**: *Staphylococcus aureus*
Bs: *Bacillus subtilis*; **Ca**: *Candida albicans*
Inhibition zone:
IZD: 10-14 mm = intermediate activity, 15mm > high activity.

Table 3: Antimicrobial activity of antibiotics on different micro-organisms (Minimum Inhibition zone in mm)

Standard Drug	Ec	Ps	Sa	Bs	Ca
Ampicillin	19	22	24	21	16
Cephalexin	-	19	21	20	15
Amoxicillin	16	15	22	25	18

Table 4: Results of Antioxidant activity of root extracts of *Citrus paradisi, C. sinensis and C. aurantium*

Root sample	Extract	%RSA ±SD (DPPH)
Citrus Paradisi	Pet. Ether	7± 0.04
	Chloroform	27± 0.03
	Methanol	52± 0.02
Citrus sinensis	Pet. Ether	10± 0.05
	Chloroform	52± 0.03
	Methanol	80± 0.02
Citrus aurantium	Pet. Ether	19± 0.02
	Chloroform	49± 0.01
	Methanol	42± 0.06
Standard	Propyl Gallate	94± 0.01

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