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Investigation Anticancer and Antimicrobial Activity of *Hibiscus sabdariffa* Water Extract

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ABSTRACT

To facing the growing need for new drugs, the investigation of medicinal plant continuing. So that in the current study, the antibacterial and antifungal and anticancer effect of *H. sabdariffa* water extract were studied**.** The antibacterial test done against *Escherichia coli*, *streptococcus mutans* and *Pseudomonas aeruginosa* were it gave the best inhibition zone 35 mm against *E. coli.* Furthermore the antifungal activity tested against *Rhizoctonia solani*, *Fusarium graminearum* and the best inhibition growth activity found against *R. solani* by 69%. The anticancer activity tested by MTT test against SR (lymphoma) and MCF7 (breast cancer) cell lines, the best cytotoxicity found against MCF7 where it reach 64%. In conclusion, the H. sabdariffa water extract have a promising properties in the discovery new drugs due to its biological activity and its viability worldwide.

Keywords: *H.sabdariffa* water extract, anticancer, antibacterial, antifungal

Introduction

Medicinal plants gained great attention in last two decades due its high biological activity, also due the growing need for new drugs or new chemical entities in drugs discovery. Where development new drugs or novel drug is very complex process, time consuming and very expensive. For all this reasons, focusing on medicinal plants will be continue in near future (Harvey, 2008). *H. sabdariffa* belongs to species hibiscus, known as Rosella and karkade, is well known plant in Africa and Asia where it seed used for oil also as medicine plant, as tea or cold drink, also its leaves used in meal and in food industry (Ali *et al.*, 2005). Nowadays, its cultivate in both tropical and subtropical regions, in Sudan, Saudi Arabia, Malaysia, Egypt, China, Mexico and Iraq also in many other countries, where it's easy to grow up and its lot of benefits. The calyx leaves are important parts and are rich in their content of active chemical compounds such as proteins, carbohydrates, fats, fiber, thaimine, niacin, riboflavin, ascorbic acid and vitamin E, as well as the elements nitrogen, phosphorus, potassium, iron, zinc, calcium and manganese (MIZUKAMI *et al.*, 1992). In folk medicine, karkade used to treat hypertension and hyperlipidemia also for their cooling effect (Wahabi *et al.*, 2010; Hopkins *et al* 2013). Phytochemical screening for *H. sabdariff* revealed presence of vital vitamins and minerals. biologically active chemical includeing: tannins, saponins, glycosides, phenols, and flavonoids (Nkumah, 2015). For these reasons, antibacterial and antifungal activity of *H. sabdariffa* were under investigation, furthermore anticancer properties, in the present study.

Material and methods

Plant Collection and Extraction

The calyces of plant obtained dried from the local market of Al-Samawwa city. Smash it by electric grinder to have powder, then 50 g from this powder dissolved in 300 ml hot water and shacked from time to time. After three days the crude extract filtered and placed on oven at 40 C̊ until complete dryness (Handa *et al.*, 2008). Five concentration were prepared for tests (200, 100, 50, 25 and 12.5 mg/ml).

Anticancer test

Two cancer cell line were used in this study, SR (lymphoma) and MCF7 (breast cancer) to evaluate the anticancer effect of *H. sabdariffa* water extract. The cells were cultured according to (Freshney, 2015) in 75 cm^2 tissue culture flasks under humidified 5% CO2 atmosphere at 37°C in RPMI-1640 medium (Sigma chemicals, England) with 10% fetal bovine serum (FBS), and penicillin- streptomycin 1% (100 U/ mL penicillin and 100 µg/mL streptomycin).

The MTT assay was done to evaluate cytotoxicity effect of the extract where cell lines treated with five concentrations (200, 100, 50, 25 and 12.5 µg/ml) for 48 h., then incubated with 10µL of MTT (37 ◦C, 2 h). After the incubation, media in the cells was aspirated, followed by addition of DMSO (100 µL) in each well. The absorbance was recorded in a micro titer plate reader at 570 nm.

Antibacterial test

For each bacteria (*Escherichia coli*, *streptococcus mutans* and *Pseudomonas aeruginosa*) 1* 10^8 cell/ml were calculated by McFarland standard, then 0.1 ml from each suspension taken and spread on Muller Hinton agar using class spreader. To study plant extract bacteria, filter paper disks prepared and saturated with the prepared concentrations of plant extract, by added 0.5 ml from extract to 10 sterilized disks in container. After that the culture incubated at 37º C for 24h. and the inhibition zone (IZ) were taken by diameter ruler for each extract and concertation.

Antifungal test

The identified fungi *Rhizoctonia solani* and *Fusarium graminearum* (obtained from the Department of Plant Protection / College of Agriculture / Al-Muthanna University) by the pure culture method, cultured by taken a piece of 0.5 cm from the end of the fungal growth and placed in the middle of the plate containing different concentrations of extract. Also, control dishes containing maximum fungus growth without extract were prepared, and all the dishes are incubated in the incubator at 27º for a period of 3-5 days, or they incubated until the fungal growth in the comparison dishes reaches the edge of the dish. After that, fungal growth rate is calculated by taking the average diameter of the colony in two orthogonal directions, and the percentage of inhibition is calculated by the following equation according to (Naeini *et al.*, 2009).

Inhibition rate = Control Growth rate- Treatment Growth rate/Control Growth rate *100

Results and discussion

In the current study, the biological activity of *H. sabdariffa* were investigated, where the results of anticancer activity were showed in Table (1), the data showed dose-depending

effect, where the high cytotoxicity 58% and 64% appeared in high concentration 200 µg/ ml for the SR and MCF7 cell lines, respectively.

Table 1. Cytotoxicity of *H. Sabdariffa* Water Extract Against SR and MCF7 cell lines

concentration µg/ ml	Cytotoxicity%	
	SR	MCF7
200	58%	64%
100	40%	49%
50	31%	28%
25	14%	25%
12.5	10%	20%

The antibacterial activity of *H. sabdariffaalso* screened against some bacteria species as shown in Table (2), and it's found to be high activity in dose-depending manner where high concentration of extract 200 mg/ml gave inhibition zone 35, 33 and 28 mm against *E. coli*, *S. mutans* and *P. aeruginosa*, respectively.

Table 2. Antibacterial Activity of *H. Sabdariffa* Against Some Bacteria

Concentration mg/ml	Inhibition zone (mm)		
	E. coli	*S. mutans*	*P. aeruginosa*
100	35	33	28
50	30	31	25
25	27	26	22
12.5	25	23	19
6.26	21	20	15

Furthermore, the antifungal activity of water extract of *H. sabdariffa* determined by examined it against two fungi, also there is a dose-depending manner in extract activity as appear in Table (3). Where it gave inhibition in growth by using 200 mg/ml where reach to 55% and 69% against *F. graminearum* and *R. solani*, respectively.

Table 3. antifungal activity of *H. sabdariffa* against *F. graminearum* and *R. solani*

Concentration mg/ml	Inhibition rate for *F. graminearum*	Inhibition rate for *R. solani*
200	55%	69%
100	30%	25%
50	14%	18%
25	12%	10%
12.5	7%	8%

The results of current study agreed with previous studies where they found antibacterial activity of the *H. sabdariffa* extract (Liu *et al.*, 2005) against several bacteria including

methicillin-resistant S*taphylococcus aureus*, *Actinetobacter baumannii* and *Klebsiella pneumonia*. In addition, the anticancer activity of H. sabdariffa extract were studied in different research and they found a high anticancer property , where it's found to inhibit the growth of prostate cancer, gastric carcinoma, skin tumor and leukemia (Tseng *et al.,* 1996; Lin *et al.,* 2005; Lin *et al.,* 2012). These effects thought to be related to high containing of phenolic compounds and anthocyanins, where they responsible for reducing reactive oxygen species (ROS), G1 arresting, DNA fragmentation and apoptosis both intrinsic and extrinsic.

Conclusion

In the current study, antibacterial, antifungal and anticancer activity of *H. sabdariffa* water extract were investigate. The results of tests demonstrate that the plant have high biological effect in high concentrations while low concentration show weak effect in different tests. Further studies will be established to reach the exact mechanisms by which the plant extract showed its effects.

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