RESEARCH ARTICLE

Antimicrobial and Immunoprotective Potentials of *Ruta chalpensis* Methanolic Extract: *In-vitro* and *In-vivo* Studies

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ABSTRACT

Plants used in traditional medicine contain a vast array of substances that can be used to treat chronic and infectious diseases. It was estimated that about 25% of all modern medicines are directly or indirectly derived from higher plants. Indeed, well into the twentieth century, much of the pharmacopeia of scientific medicine was derived from the herbal lore of native people. *Ruta chalepensis* are plants used in folkloric medicine as antispasmodics, digestive, and for intestinal gases. *R. chalepensis*, is a species of the family Rutaceae. The species is hairless, not glandular at the top, 30 to 80 cm with broad leaves. This study aimed to investigate the *in-vitro* (antimicrobial) and *in-vivo* (immunoprotective activity) of *R. chalepensis* methanolic extract. The Results indicated the plant's capacity to inhibit microbial growth against *Eschereshia coli, Staphyloccocus aureus*, and *Candida albicans* and stimulate immunoglobulin titer in mice damaged with CCL_4 in comparison with positive and negative controls.

Keywords: *Candida albicans*, *Eschereshia coli*, Immunoglobulin, *Ruta chalepensis, Staphyloccocus aureus*.

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INTRODUCTION

In the latest years an excessive scientific improvement concerns pharmacological and chemical studies of medicinal plants finding new compounds with biological properties.[1] Indeed, the presence of secondary metabolites that support health and decrease infection has been very interesting in all worlds. The importance of herbs used in the original system of medicines to control the body's immune system.[2] Plants produce a wide diversity of secondary metabolites (SM), which serve as defense compounds against herbivores, other plants, and microbes, and signal compounds. Because of this, some plants or products isolated from them have been and are still used to treat infections, health disorders, or diseases. Phytochemicals compound such as polysaccharides, flavonoids, peptides, natural sulfur compounds, and tannins are "known to moderate the immune system *in-vivo*". Rutaceae, generally identified as citrus family, is part of flowering plants with approximately 160 genera.[3] Among the innumerable species of therapeutic attention, plants are going to the Rutaceae family, «which has species of financial, biological and therapeutic significance which is *R. chalpensis*.[4] The phytochemicals of *R. chalpensis* characterized the presence of volatile oil, "flavonoids, alkaloids, coumarins, "flavonoids, "glycosides and tannins are reflected the potential inhibitors of pro-inflammatory molecules.[5] *R. chalpensis* widely used in the Mediterranean area to treat pain, dermatitis, rheumatism, and other inflammatory diseases.[6] *R. chalpensis* possessed antibacterial properties in methanol extract of different plant parts (leaves, flower, and stem) against highly pathogenic bacteria such as *S. aureus, E. coli* in addition to inhibiting the *in-vitro* growth of *C. albicans.*[7,8]

METHODS

Plant Sample Collection and Identification

An amount of 250 g of *R. chalepensis* plant greeneries was collected from the local markets of Baghdad, Iraq. The leaves, previously identified by National Herbarium of Iraq, were ready, dried, and set in the shadow at room temperature, then grounded and prepared for extraction.

Methanolic Abstraction

Some 30 g of punished plant leaves were balanced and allocate "thumble tube in soxhlet device. Aliquots " of 200 mL from methanol alcohol were used for extraction at 45°C for 7 hours; after that, the solvent was evaporated by rotary evaporator. The residue was put in an incubator at 37°C until the solvent fully disappeared. After that, the extraction was calm and weighed formerly diluted with distilled water to make the required concentrations, and then the extract was sterilized with "Millipore filter paper (0.22 mm).[9]

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Microbial Isolates

E. coli, *S. aureus*, and *C. albicans* were obtained from the microbiology Lab, "Biotechnology Research Center, Al-Nahrian University, Iraq" where they were detected beforehand. The estimated bacterial groups where collected by using serial dilutions to obtain "108 × 10" CFU/mL.

Research Laboratory Faunas

This study required albino male mice (swiss), which were obtained from "Biotechnology Research Center (Al-Nahrain University)". Their time of life ranges amid 8–9 weeks, and their heaviness was 23–27 grams at the starting of the research. They were captive in the animal household of the provider, in which the temperature was 24–27ºC, and a sunlit: dim periods of "10:15 h/d." The animals had free excess to diet (standard pellets) and intake water through all research.

EXPERIMENTAL PROJECT

Primary Step

To investigate the inhibitory effect against *E.coli, S.aurous* and *C. albicans*."Three concentrations (50, 100, 150 mg/mL) of methanol extract of *R. chalpensis* greeneries were ready.

Preparation of the Microbial Isolates

The suspensions of bacteria and yeast were prepared by inoculating their cultures in test tubes having 10 mL of "NB for bacteria and "PDB for yeast continuously; after 19 hours of incubation at 37°C, duplicate serial dilution was prepared for culture and read with a Spectrophotometer at wavelength 420 nm.

R. chalpensis Methanolic Extract Inhibitory Effect against Tested Microorganism

The method was used to identify the inhibitory result of plant extract in contradiction of bacteria and yeast was Agar-Well Diffusion, by creation 6 mm diameter wells using pasture pipette on the agar media, then 0.2 mL of each concentration "(50,100 and 150 mg/mL) of plant extract were put in each well, excluding one filled with distilled water as a negative control then 0.1 ml of bacteria and yeast suspension was added to each well and kept back in the incubator for "24 hours" to diffuse, after incubation at 37ºC for 24 hours where the inhibitory result of plant extract was determined by measuring the diameter of inhibition zone around each well.[10]

Second Stage (Immunological Assessments)

In order to investigate immunological parameters, six mice groups were used:

- *Group I:* mice not treated with any material (negative control)
- *Group II:* mice treated with 0.02% CCL_4 (positive control)
- *Group III:* mice treated with first dose of plant extract at 50 mg/kg
- *Group IV:* mice treated with CCL4 in day 1 and from 2 to 7 days with plant extract (50 mg\kg)
- *Group V:* mice treated with second plant dose (100 mg\kg)
- *Group VI:* mice treated with CCL4 in day 1 and from 2 to 7 days with plant extract (100 mg\kg)

To evaluate the concentration of all immunoglobulin, mice injected intraperitoneally 0.1 mL of tested material for 7 days and sacrificed on day 8th then blood was collected by heart puncture, transferred to Eppendorf tube and allowed to clot at room temperature for 15 minutes, and then serum was separated by centrifugation at 3,000 rpm for 10 minutes. That collected serum was used for the assessment of immunoglobulin titer. To carry out these assays determined in mouse serum by following the enzymatic colorimetric method,[11] commercial kits (Randox Company) were used.

Statistical Analysis

Data in this study were presented as mean ± SD. The statistical programme GraphPad Prism version 5.01 (Graph Pad software, Inc., La Jolla, CA, USA) and statistical analysis system—SPSS version 14 were used. Differences between means were considered by Duncan's test, in which $p \leq 0.05$ was significant.[12]

RESULTS AND DISCUSSION

Antimicrobial Influences

The plant extract exhibited significant inhibitory effects on bacteria and yeast growth", as shown in Table 1. The plant extract (150 mg/mL) caused a significant growth inhibition than other concentrations on *E. coli* and *S. aurous* reaching 20.25 ± 0.22 and 22.25 ± 0.19 mm, correspondingly while the concentration (150 mg/mL) caused a significant growth inhibition to *C. albicans* reaching 17.82 ± 0.11 mm. (Table 1)

Immunological Activity

The results of immunoglobulin study (IgG, IgM and IgA) as shown in Table 2 indicated that mice treated with plant possess the ability to enhance the concentrations of IgG and IgM

Table 1: Antimicrobial Influences

Bacterial type and yeast	*Ruta chalpensis concentration*		
	50 mg/mL	*100 mg/mL*	*150 mg/mL*
E.coli	16.35 ± 0.37	17.71 ± 0.22	20.25 ± 0.22
S. aurous	19.35 ± 0.34	20.75 ± 0.12	22.25 ± 0.19
C. albicans	14.35 ± 0.34	15.18 ± 0.31	17.82 ± 0.11

Table 2: Immunoglobulin study (IgG, IgM and IgA)

Groups	*Dose (mg/kg)*	*IgG (mg/dL) Mean ± S.E.*	*IgM (mg/dL) Mean ± S.E.*	*IgA (mg/dL) Mean ± S.E.*
Negative control	—	1660 ± 17.2	50.5 ± 2.8	71.8 ± 5.3,
Positive control	0.02	50.5 ± 4.8	45.3 ± 4.7	222.2 ± 23.6
R. chalepensis	50	2729 ± 25.6	226.5 ± 14.6	—
CCL4 + *R. chalepensis*	50	945.1 ± 23.9	45.6 ± 6.9	203.7 ± 27.8
R. chalepensis	100	3812.8 ± 42.3	498.5 ± 6.9	—
CCL4 + *R. chalepensis*	100	1562.1 ± 38.1	50.3 ± 6.2	87.7 ± 6.9

immunoglobulin in the two doses tested in comparison with controls (2729 ± 25.6, 226.5 ± 14.6 mg/dL) and (3812.8 ± 42.3, 498.5 ± 6.9 mg/dL) for 50 and 100 mg/kg, respectively in compared to negative and positive control which possess the immunoglobulin titer of 1660 ± 17.2, 50.5 ± 2.8 mg/dL and 50.5 ± 4.8, 45.3 ± 4.7 mg/dL, respectively while in interaction groups (IV and VI) reached to (945.1 ± 23.9, 1562.1 ± 38.1 mg/dL) and 45.6 ± 6.9 and 50.3 ± 6.2 mg/dL for IgG and IgM respectively. The IgA concentration in control negative and positive mice reach to 71.8 ± 5.3, 222.2 ± 23.6 mg/dL respectively, while in interaction groups (IV and VI) reach to 203.7 ± 27.8, 87.7 ± 6.9 mg/dL, respectively.

DISCUSSION

Today the medications and nutritional supplements factories pay special attention to natural resources and tend to use much local flora into the production. Besides that, efforts have been gradually more to use herbal drugs as a substitute medicine. The great inhibitory consequence of methanol extract of *R. chalpensis* might remain because of the capacity of alcohol to extract all or greatest of dynamic complexes found in plant leaves, counting glycosides, tannins, and cumarins flavonoids, primarily rutin and quercetin, which characterize one of the most abundant ordinary flavonoids. This result reaches agreement with other previous researches[13] who originate that methanol extraction is an exemplary method to gain active components from *Cassia italica* leaves flavonoids particularly. The primary role of flavonoids on microorganisms and yeast is by creating multiplexes with cell proteins and liquefied proteins that will interrelate with bacteria cell wall affecting its osmosis and ultimately leading to cell death. It will incorporate with bacterial deoxyribonucleic acid (DNA) and negatively influencing its vital activities.[14] Flavonoids, tannin, saponin, and triterpenic acids exhibited good antimicrobial residences in opposition to both gram-positive and gram-negative microorganisms' longevity. The methanolic extract in the above experiment indicates high flavonoids which have good antimicrobial activities. This activity may be linked to the presence of essential oil compounds which affect many bacteria and yeast in varying degree.[15,16]

R. chalepensis has been reported to contain alkaloids, phenolics, flavonoids, and flavanol compounds. Furthermore, γ-fagarine and kokusaginine have various health benefits, including antioxidant, cytotoxicity, antibacterial, antifungal, immunological, and other biological activities It was also of interest to note that *R. chalepensis* methanol extract exerted some immunological modulation and anti-inflammatory effects in mice. Immunoprotective effects of the extract were overwhelmed by inducing granulocyte-macrophage colony-stimulating factor, macrophage colony-stimulating factor, interleukins IL-2 IL-4 and IL-5 regulate the proliferation, differentiation and maturation of committed stem cells responsible for the production of white blood cells.[17] Such enhancement, because the phytocompounds in the extract stimulated the production of these regulatory factors or increased the sensitivity of the committed stem cells, responsible for the production of white blood cells.[18,19] Accordingly, theimmuno enhancement can be ascribed to these chemical constituents (flavonoids and tannins), which may modulate the immune response through the interaction between cytokines affected by the plant extracts treatment.[20-22]

REFERENCES

1. Günaydin, K. and Savci, S. (2005) Phytochemical studies on *Ruta chalepensis* (Lam.) Lamarck. Nat. Prod. Res. 19:203-210.
2. Ad'hiah, A. H., Ibraheem R. M. and Abbood, K. W. Modulation of cytokine production from cultured mononuclear cells of leukemia patients by Hypericum triquetrifolium Turra methanolic extract. Arab Journal of Basic and Applied Sciences. 2018;25(1):13-19.
3. Kacem, M., Kacem, I., Simon, G., Ben Mansour, A., Chaabouni, S., Elfeki, A., Bouaziz, M. 2015. Phytochemicals and biological activities of *Ruta chalepensis* L. growing in Tunisia. Food Biosci. http://dx.doi.org/10.1016/j.fbio.2015.08.001.
4. Dob, T., Dahmane, D., Gauriat-Desrdy, B., Daligault, V. 2008. Volatile Constituents of the Essential Oil of *Ruta chalepensis* L. *sub* sp. *Angustifolia* (Pers.) P. Cout. J. Essent Oil Res. 20(4): 306-309.
5. Mejri J, Bouajila J, Sik Ali SB, Abderrabba M, Mejri M. 2012. *Ruta chalepensis* L. Essential Oil: Chemical Composition and Phytotoxic Activity. JBAPN, 2(6):341-352.
6. Akkaria, H., Ezzineb, O., Dhahri, S., B'chir, F., Rekik, M., Hajaji, S., Aziz Darghouth, M., Lahbib Ben Jamâa, M., Gharbi, M. 2015. Chemical composition, insecticidal and in vitro anthelmintic activities of *Ruta chalepensis* (Rutaceae) essential oil. Ind Crop Prod. 74:745-751
7. Pino O, Sánchez Y, Rojas MM, Abreu Y, Correa T, Martínez D, Montes de Oca R. Composición química y actividad antibacteriana del aceite esencial de Ruta chalepensis L. Rev Protec Veg. 2014;29(3):220-225.
8. Schmelzer GH, Gurib-Fakim A. Plant resources of tropical Africa. Medicinal Plants. 2013;2(11):2:417.
9. Simandi B, Kery A, Kristo ST, Andras C, Prechi A. and Fekete J. Supercritical fluid extraction of non-volitile terenoids from medicinal plant. Acta. pharma hung., 2001;71:318-324.
10. Al-Naimy, E. H., Al-Amery, M. M., Al-ezzzy R. M. Antibacterial and Cytogenetic Effect of Cassia Italica Leaf Extract on Albino Male Mice. Journal of Al-Nahrain University, 2010;13(10): 132-139.
11. Reitman S, and Frankel S. "A Colorimetric Method for the Determination of Serum Glutamic Oxalacetic and Glutamic Pyruvic Transaminases." Am J Clin Pathol, 1957;28:56-63.
12. Steel RGD, and Torri. Principles and procedure of statistics. Mg.Hill book company. INC. New York, Toronto, London. 1960;481.
13. Jamel J, Bouajila J, Aydi A, Barth D, Abderrabbaa M, and Mejri M 2012 Supercritical CO2 extract and essential oil of Ruta chalepensis L Growing in Tunisia: A natural source of Undecan-2-one Analytical Chemistry Letters 2(5):290.
14. Gibka J, Kunicka-Styczyñskab A and Gliñski M 2009 Antimicrobial activity of Undecan-2-one, Undecan2-ol and their derivatives Journal of Essential Oil Bearing Plants 12(5):605.
15. Albado E, Saez G, and Grabiel S 2001 Chemical composition and antibacterial activity of the essential oil of Origanum vulgare Heredian Medical Review 12(1).
16. Henao J, Muñoz L, Padilla L and Ríos E 2010 Extraction and characterization of the essential oil of H.B.K "orégano de monte" cultivated at Quindío and evaluation of antimicrobial activity Revista de Investigaciones de la Universidad del Quindío 21 82.

17. Al-Ezzy, R. M., Mohammed Hassan, Z. Y., and Al-Jumaili, F. T. O. 2017. "Hematological Toxic Effect and the Frequency of Micronucleus Formation of Different Doses of Cyproheptadine on Albino Male Mice Blood Picture." Iraqi Journal of Hematology 37:149-153
18. Khalaf, H. M. Detection of the organs protective activity of ethanolic extract of cyperus rotundus on albino male mice damaged by methotrexate drug. Plant Archives Vol. 20 Special Issue, 2020;3021-3026.
19. Kuttan G. Immunomodulatory effect of some is naturally occuring sulphur containing compounds. J Ethnopharmacol 2000;72:93-99.
20. De Freitas TG, Augusto PM, Montanari T. Effect of Ruta graveolens L. on pregnant mice. Contraception. 2005;71(1): 74-77.
21. Al-Ezzy, R. M., Al Anee, R. S. A., and Ibrahim, N. A. Assessments of Immunological Activity of Achillea Millefolium Methanolic Extract on Albino Male Mice." Journal of Pharmacy and Pharmacology,2018;6:563-569.
22. Hasan,Z.Y.M., Al-Ezzy, R.M., Abdullah, J.M. and AL-Hamdi, B.S. Immunomodulation of cyproheptadine hydrochloride on albino mice blood lymphocytes. Iraqi Journal of Hematology IP: 37.239.154.122.