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Estimation of the total flavonoid, antioxidant, anti- bacterial potentials of *Rutachalepensis* methanolic extract

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ABSTRACT: The objective concerning this study was to decide aggregation flavonoid content (TFC) primarily based of flavonoid-aluminum chloride (AlCl3) and antioxidant activity by spectrophotometric method and determination of against bacterial action in *rutachalepensis* (natural plant) utilizing reaction surface philosophy*rutachalepensis* has. considerable cell antioxidant properties. The among invitro records evidently portrayed the most cancers prevention agent adequacy of methanolic extricate, which was tried. spectrophotometrically strategy is a vital procedure to decide add up to total flavonoids, and cancer prevention agent action (reductive capacity and DPPH radical foragers action). Results showed that rutachalepensis has high flavonoid substance which was (257± 0.280 µg/ml) in addition to tremendous antioxidant in a concentration dependant manner(0.293± 0.01,0,743± 0.013 for 0.558, 0.02 and 0.64 mg/ml respectively for reductive ability and 68.60to 85.03 for 0.125 and 0.500 mg/ml in DPPD radical scavenging activity and antibacterial in opposition to Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli. Key word: *rutachalepensis* , herbal remedies, healthcare, annual plant

I. Introduction

The vast majority of people on this planet still rely on their traditional *materiamedica* (medicinal plants and other materials) for their everyday health care needs. It is also a fact that one quarter of all medical prescriptions are formulations based on substances derived from plants or plant-derived synthetic analogs, and according to the World Health Organization (WHO), 80% of the world's population, primarily those of developing countries, rely on plant-derived medicines for their healthcare (1,2). *Rutachalepensis*belongs to *Rutaceae* family is an aromatic herbs that growing up to 80 cm in height(3).The aerial parts of plant is used as treatment of rheumatism, an analgesic, antipyretic and for mental disorder. Its oily compound have many uses such as stimulate nervous system and uterine(4). From the fresh leaves, juice obtained is given to children for many such as helminthic infection, decrease otalgia and odontalgia. properties of these plants(5). Essential oils are the most interesting components of herbals and plants. These compounds are rich in hydrocarbon that have oxygenated, hydrogenated, and dehydrogenated functional groups. Most of these chemicals are monoterpenoid or sesquiterpenoids. They are odorous principles, which are found in various plants parts and evaporate at ordinary temperature(6,7).

II. Material and method

2.1- plant collection and identification.

Rutachalpenesis collected from Baghdad Iraq during period march 2019 , then it dried at room temperature after that grinding plant with grinding device.

2.2-Preparation of Plant Extract

Methanolic extract of *Rutachalpenesis*was prepared according to(8). Fifty grams of the plant leaf powder were extracted with 80%methanol (250 ml) at 65°C for 3 hours using the soxhlet apparatus. The extract solution was concentrated to drynessunder reduced pressure in a rotary evaporator to yield dried crude extract, which was frozen at -20°C until use to prepare the required doses and concentrations.

2.2-Determination of Total Flavonoids

Total flavonoids content was spectrophotochemically determined in the methanolic extract of *Rutachalpenesis*as rutin (flavonoids standard) equivalent by aluminium chloride colorimetric method as described by (9). The methanolic extract (3.2 mg) was dissolved in 5 ml of 50% methanol, followed by addition of 1 ml of a 5% (w/v) sodium nitrite solution. After 6 min, 1 ml of a 10% (w/v) aluminium chloride solution was added and the mixture was allowed to stand for a further 5 minutes before 10 ml of a 10% (w/v) NaOH solution was added. The mixture was made up to 50 ml with distilled water and mixed well. Then the absorbance was measured at 450 nm with a spectrometer after 15 min. A similar procedure was applied to six concentrations (2.5, 5, 10, 20, 40 and 80 µg) of rutin, and from which a standard curve was prepared to calculate the total flavonoid of plant.

2.3-Assessment of Anti-oxidant Activity *in vitro*

Anti-oxidant activity of the*Rutachalpenesis*methanolic extract was *in vitro* assessed through two evaluations, which were reductive ability and DPPH radical scavenging activity.

2.3.A-Reductive Ability

The method described by(9) was adopted to evaluate the reductive ability, in which 1 ml of each concentration of the *Rutachalpenesis* (0.02, 0.04, 0.08, 0.16, 0.32 and 0.64 mg/ml) was mixed with 1ml of 0.2M phosphate buffer (pH 6.6) and 1.5 ml of 1% potassium ferricyanide, and then incubated at 50°C for 20 minutes. Then, 1ml of 10% trichloroacetic acid was added to the mixture to stop the reaction. The mixture was centrifuged for 10 minutes at 3000 rpm, and 2.5 ml of the supernatant was mixed with 2 ml of distilled water and 0.5 ml of freshly prepared 1% Ferric chloride.

2.3.B-Evaluation DPPH Redical Scavenging Activity.

The antioxidant activity of plant methanolic extract and standard (vitamin C) were assessed on the basis of the radical scavenging effect of the stable DPPH free radical, and the method of (10) was followed. An aliquot of 0.1 ml of the extract or standard (0.625, 0.125, 0.250 and 0.500 mg/ml) was added to 3.9 ml of DPPH solution in a test tube. After incubation at 37°C for 30 minutes, the absorbance of each solution was determined at 517nm using spectrophotometer. All measurements were made in triplicates. The ability to scavenge DPPH radical was calculated by the following equation:

$$\text{DPPH radical scavenging activity}\,(\%) = \left(1 - \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}}\right) \times 100$$

2.3.C-Source of Bacteria Isolate and Identification

Two sources for bacteria isolated had been used 1.urnary tract infectionpatients in Baghdad hospital \Baghdad \Iraq for staph.aureus E.coli and . 2. pseudomonas. was isolated from patients with cystic fibrosis . After preparing bacterial media according to (11),single colonies from each type of bacteriaindicated above were grown on nutrient agar for 18-24 hrs and transferred to tube containing 5ml of Normal saline and mixed well by vortex, then bacterial growth was compared with McFarland tube. The turbidity of standard solution tube number was equivalent to a bacterial inoculums concentration of 1.5×10^{8} cell/ml. By using cotton swab, a touch of bacterial culture from normal saline was transferred to Muller Hinton agar prepared above and streaked three times by rotating the plate approximately 60°between the streaking, to ensure even distribution of the inoculums , the inoculated plates were placed at room temperature for 10 min to allow absorption of excess moisture.Then , by using sterilized Pasteur pipette each wells which were filled with 100 µl of *Rutachalpenesis*methanolic extract with different concentration (100, 200 and 300 mg/ml) and the plates were incubated at 37ºC for 18-24 hours. After incubation, inhibition zone were measured by ruler to determine their diameters in millimeters, then the results were recorded(14).

Statistical Analysis

The values of the investigated parameters were given in terms of mean ± standard error (SE), and differences between means were assessed by analysis of variance (ANOVA)

III. Results and Discussion

3.1 Determination of Total Flavonoids

Total flavonoids content were spectrophotochemically determined in methanolic extract of *Rutachalpenesis* as rutin equivalent. The extract was found to contain 257± 0.280 µg/ml flavonoids. Such finding is in a good agreement with a study carried out by 15 in which it was demonstrated that *Rutachalpenesis* grown in Iraq is a rich source of flavonoids such as rutin, hyperoside, apigenin-7-O-glucoside, kaempferol, quercitrin, quercetin and amentoflavone. Flavonoids have attracted considerable interest as dietary constituents and the results of clinical studies have indicated their possible role in preventing cardiovascular diseases and several kinds of cancer (16). Furthermore, varieties of flavonoids including apigenin, luteolin and quercetin were found to inhibit NO production through downregulating iNOS induction (17). Further studies revealed that natural products such as flavonoids and phenolics have been observed to be efficient free radical scavengers and lipid peroxidation inhibitors (18), and probably, the best described and most useful property of almost every group of flavonoids is their capacity to act as antioxidants; protecting the body against reactive oxygen species 14).

3.2- Reductive Ability

In all concentration tested (0.02, 0.04, 0.08, 0.16, 0.32 and 0.64 mg/ml), the absorbance of*Rutachalpenesis*methanolic extract was significantly higher ($P \leq 0.001$) than trolox (vitamin E), and such findings suggest that the plant extract is more effective than trolox in the reductive ability, which was concentration-dependent. It was 0.293 ± 0.010 at the concentration 0.02 mg/ml of the methanol extract, and increased significantly ($P \leq 0.05$) to 0.743 at the concentration 0.64 mg/ml ,this approach using according to (8)

Table 3.1: Reductive ability of *Rutachalpenesis*methanolic extract and trolox (vitamin E)

Concentration (mg/ml)	Reductive Ability Absorbance (Mean ± SD)	
	*Rutachalpenesis*Extract	Trolox (Vitamin E)
0.02	0.293 ± 0.010^{E}	0.100 ± 0.001^{D}
0.04	0.392 ± 0.007^{D}	0.101 ± 0.001^{CD}
0.08	0.451 ± 0.007^{C}	0.108 ± 0.001^{CD}
0.16	0.660 ± 0.013^{B}	0.114 ± 0.004^{C}
0.32	0.668 ± 0.016^{B}	0.132 ± 0.007^{B}
0.64	0.743 ± 0.013^{A}	0.211 ± 0.015^{A}

Different letters: Significant difference ($P \leq 0.05$) between means of columns.

3.2.2 DPPH Radical Scavenging Activity

Methanolic extract of *Rutachalpenesis*was significantly more effective in DPPH radical scavenging activity than vitamin C at the three concentrations tested (0.125, 0.250 and 0.500 mg/ml) according to(10). The concentrations 0.250 and 0.500 mg/ml of plant extract shared an approximated higher scavenging activity (80.00 ± 2.00 and 80.66 ± 1.15%, respectivelyVitamin C also showed variations between the four concentrations but the difference was not significant.

Table 3-2: DPPH radical scavenging activity of *Rutachalpenesis*methanolic extract and vitamin C

Concentration (mg/ml)	DPPH Radical Scavenging Activity (Mean ± SD; %)	
	*Rutachalpenesis*Extract	Vitamin C
0.125	63.66 ± 3.51^{B}	41.33 ± 10.01^{A}
0.250	80.00 ± 2.00^{A}	48.33 ± 8.50^{A}
0.500	80.66 ± 1.15^{A}	53.00 ± 10.53^{A}

Different letters: Significant difference ($P \leq 0.05$) between means of columns.

3.3-Detection of antibacterial activity

The antibacterial experiment were the methanol extract of the plant concentration 100,200,300 mg/l where test against three types of bacteria (Staphylococcus aureus,Pseudomonas aeruginosa, Escherichia coli). Large inhibition zone found in S.aureus followed by E.coli, while no inhibition in P. aeruginosa. However, the inhibition zone is increase as concentration of the extract increased.

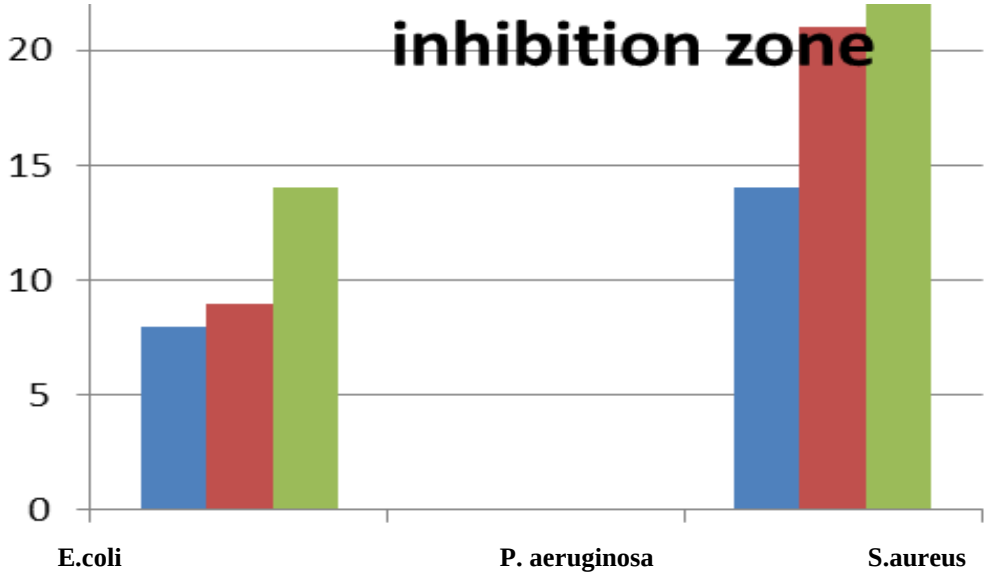

Figure 4. Inhibition zone of the three bacteria in *Rutachalpenesis*exract

The increasing in the reductive ability of *Rutachalpenesis*and DPPH radical scavenging activity in comparison with controls of each one (vitamins E and C, respectively) can be attributed to its high flavonoid content, and such finding matches with the result of methanolic extract of8*rutachalpnesis* species growing in Iraq. All the species showed a high antioxidant activity and DPPH radical scavenging activity, which was probably due to the high content of amino acids, saponins, phenols, flavonoids, alkaloids, and furocoumarins, and these constituents have been suggested to act as antioxidants (19). DPPH radical scavenging activity increased with increasing phenolic components such as flavonoids, phenolic acids and phenolic diterpenes. These phenolic components possess many hydroxyl groups including O-dihydroxy group, which have a very strong radical scavenging effect and antioxidant power (20).

It has been known that a variety of plant extract has antioxidant activities to scavenge free radicals that cause diseases via lipid peroxidation, protein peroxidation and DNA damage (21).

Phenolics are found in large quantities in the plant kingdom, and they have been proposed to have multiple biological functions, including antibacterial activity (22). Phenolics, such as flavonoids, phenolic acids, stilbenes, lignans, lignin and tannins that are especially common in leaves, flower tissues, and woody parts such as stems and barks have strong antioxidant activity (23). In our study in *Rutachalpenesis*methanolic extracts have shown antibacterial activity against Staphylococcus aureus,

Pseudomonas aeruginosa, Escherichia coli).. Our results agreement with the result of (24) who found that the main constituents of *Rutachalpenesis*methanolic extract include thymol, carvacrol and flavonoids , tannin, saponin and triterpenic acids that cause of exhibited a good antimicrobial properties against both Gram-positive and Gram-negative bacteria.

References

[1]. Agyare C, Boakye YD, Bekoe EO, Hensel A, Dapaah SO, Appiah T.2011.Review:African medicinal plants with wound healing properties. 11;177:85-100.

[2]. Ali A, Demirci B, Kiyan HT, Bernier UR, Tsikolia M, Wedge DE, Khan IA, Başer KH, Tabanca N. 2013.Biting deterrence, repellency, and larvicidal activity of Rutachalepensis (Sapindales: Rutaceae) essential oil and its major individual constituents against mosquitoes.50(6):1267-74.

[3]. Allevato DM, Groppo M, Kiyota E, Mazzafera P, Nixon KC. 2019 Evolution of phytochemical diversity in Pilocarpus (Rutaceae).Phytochemistry.May 9;163:132-146

[4]. Ntalli NG, Manconi F, Leonti M, Maxia A, Caboni P.2011.Aliphatic ketones from Rutachalepensis (Rutaceae) induce paralysis on root knot nematodes.13;59(13):7098-103.

[5]. Haddouchi F, Chaouche TM, Zaouali Y, Ksouri R, Attou A, Benmansour A. 2013.Chemical composition and antimicrobial activity of the essential oils from four Ruta species growing in Algeria.1;141(1):253-8.

[6]. AouadhiCh, Ghazghazi H, Hamrouni S, Hasnaoui B, Maaroufi A. 2013.In vitro antifungal activity of the essential oil and the methanolic extract of Rutachalepensis..Arch Inst Pasteur Tunis;90(1-4):39-46.

[7]. Bennaoum Z, Benhassaini H, Falconieri D, Piras A, Porcedda S. 2017.Chemical variability in essential oils from Ruta species among seasons, and its taxonomic and ecological significance. Nat Prod Res. Oct;31(19):2329-2334

[8]. Fua, W., Chena, J., Caia, Y., Leia, Y., Chenb, L., Peic, L., Zhoua, D., Lianga, X. and Ruana, J. (2010). Antioxidant, free radical scavenging, anti-inflammatory and hepatoprotective potential of the extract from Parathelypterisnipponica (Franch. etSav.) Ching. *J Ethnopharmacol*., 130: 521-528.

[9]. Sakanaka, S., Tachibana, Y. and Okada, Y. (2005). Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinohacha). *Food Chem*., 89: 569-575 .

[10]. Sanja, S.D., Sheth, N.R., Patel, N.K., Dhaval, P. and Biraju, P. (2009). Characterization and evaluation of antioxidant activity of portulacaoleracea. *Int. J. Pharm. Pharm. Sci*., 1: 74-84.

[11]. Santurio D. F., de Jesus F. P. K., Zanette R. A., Schlemmer K. B., Fraton A., Fries L. L. M. Antimicrobial activity of the essential oil of thyme and of thymol against *Escherichia coli* strains. *Acta Scientiae Veterinariae*. 2014;42(1):1–4.

[12]. Bouajaj S, Romane A, Benyamna A, Amri I, Hanana M, Hamrouni L, Romdhane M.2014.Essential oil composition, phytotoxic and antifungal activities of Rutachalepensis L. leaves from High Atlas Mountains (Morocco).;28(21):1910-4.

[13]. Conti B, Leonardi M, Pistelli L, Profeti R, Ouerghemmi I, Benelli G.2013 Larvicidal and repellent activity of essential oils from wild and cultivated Rutachalepensis L. (Rutaceae) against AedesalbopictusSkuse (Diptera: Culicidae), an arbovirus vector.112(3):991-9.

[14]. Günaydin K, Savci S.2005 Phytochemical studies on Rutachalepensis (Lam.) Lamarck.19(3):203-10.

[15]. Gurib-Fakim, A. (2006). Medicinal plants: traditions of yesterday and drugs of tomorrow. *Mol Aspects Med J*., 27: 91-93.

[16]. Hamdiken M, Bouhalit S, KechridZ.Effect of Rutachalepensis on Zinc, Lipid Profile and Antioxidant Levels in the Blood and Tissue of Streptozotocin-Induced Diabetes in Rats Fed Zinc-Deficient Diets. Can J Diabetes. 2018 Aug;42(4):356-364.

[17]. Al-Ezzy R. M. I., F. T. O. A.-J. a. N. K. A.-A. (2017). "Detection of Total Flavenoids, Reductive Ability, and Anti-microbial in Glycyrrhiza and Achillea Medicinal Plants." Journal of Biology and Life Science, 8(2).

[18]. Abbood ,K.W., Al-Ezzy, R. M., Ad'hiah, A. H.(2015). "Antioxidant activity of HypericumtriquetrifoliumTurra methanol extract in vitro." International Journal of Medicinal Plants (Photon)108: 632-637.

[19]. Khoury M, Stien D, Ouaini N, Eparvier V, Arnold Apostolides N, El Beyrouthy M. 2014. Chemical composition and antimicrobial activity of the essential oil of Rutachalepensis L. growing wild in Lebanon..11(12):1990-7

[20]. Lehmann H. 2015 Medicinal plants in France, between pharmacy and herb trade: historical and legislative aspects.;73(5):391-8.

[21]. Liu SM et al. J Zhejiang. (2017)Characteristic differences in essential oil composition of six Zanthoxylumbungeanum Maxim. (Rutaceae) cultivars and their biological significance.UnivSci B

[22]. N, Adwan L, K'aibni S, Zaid AN, Shtaya MJY, Shraim N, AssaliM . 2017.Variability of Chemical Compositions and Antimicrobial and Antioxidant Activities of *Rutachalepensis* Leaf Essential Oils from Three Palestinian Regions.Jaradat.Biomed Res Int.;2017:2672689

[23]. Ouerghemmi I et al.. (2017)Antioxidant and antimicrobial phenolic compounds from extracts of cultivated and wild-grown Tunisian Rutachalepensis.Volume 25, Issue 2350-359,

[24]. Kacem M, Simon G, Leschiera R, Misery L, ElFeki A, Lebonvallet N.2015. Antioxidant and anti-inflammatory effects of Rutachalepensis L. extracts on LPS-stimulated RAW 264.7 cells..In Vitro Cell Dev Biol Anim. Feb;51(2):128-41