

ESTIMATION OF THE WHOLE FLAVONOID, ANTIOXIDANT, ANTI BACTERIAL CHALLENGE CONCERNING *VIOLA ODORATA* (BANAFSHA) METHANOLIC EXTRACT.

R. M. Ibraheem
Lecturer

A. A. Mhawesh
Lecturer

K. W. Abood
Prof.

Coll. of Biotech.- Univ. of Al-Nahrain Coll. of Biotech.- Univ. of Al-Nahrain Coll. of Biotech.- Univ. of Al-Nahrain

ABSTRACT

The objective concerning this study was to decide aggregation flavonoid content (TFC) primarily based of flavonoid-aluminum chloride ($AlCl_3$) and antioxidant activity by spectrophotometric method and determination of against bacterial action in *Viola odorata* (natural plant) utilizing reaction surface philosophy. Homegrown prescription or restorative plants have prompted the disclosure of various new medications, then non-tranquilize substances. Among it flora are kinds of the range group *Viola* has a region including family violaceae then accommodates round 19 species of comparable sort. *Viola odorata* (Banafsha) has considerable cell antioxidant properties. The among vitro 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 *Viola odorata* has high flavonoid substance which was $(341 \pm 0.280 \mu\text{g/ml})$ in addition to tremendous antioxidant in a concentration dependant manner (ranged from 0.170 to 0.498 for 0.02 and 0.64 mg/ml respectively for reductive ability and 70.60 to 87.03 for 0.125 and 0.500 mg/ml in DPPD radical scavenging activity) and antibacterial in opposition to (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* or *Streptococcus*).

Key word: herbal remedies, healthcare, annual plants, inhibition zone .

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قياس الفلافونويدات الكلية، التأثير المضاد للأكسدة، الفعالية المثبطة لنمو البكتريا للمستخلص الميثانولي لنبات الفيولا العطرية (البنفسج).

خلود وهيب عبود
استاذ

احمد علي مهاوش
مدرس

رقية محمد ابراهيم
مدرس

كلية التقنيات الاحيائية اجماعه النهريين

كلية التقنيات الاحيائية اجماعه النهريين

كلية التقنيات الاحيائية اجماعه النهريين

Khulood_whyeb@yahoo.com alshammariamhmed.a.m@gmail.com Ruqaiya.alezzy83@yahoo.com

المستخلص

ان الهدف من هذه الدراسة هو قياس المحتوي الكلي للفلافونويدات علي أساس تفاعل الفلافونويدات مع كلوريد الالمنيوم وتحديد النشاط المضاد للاكسده بواسطة طريقه قياس امتصاص الطيف الضوئي وتحديد الفعالية المضادة للبكتريا باستخدام منهجيه سطح الاستجابة. أدت الادويه العشبية أو النباتات الطبية إلى اكتشاف انواع جديدة وغير جديدة من الادوية. من بين هذه النباتات أنواع من جنس فيولا ينتمي إلى اسره البنفسج ويشمل حوالي 19 نوعا من جنس واحد. فيولا اودوراتا (بانافشا) تمتلك فعالية عالية ضد المواد المؤكسدة والجذور الحرة والتي اكدتها الفحوصات المختبرية التي اجريت على المستخلص الميثانولي الذي تم اختباره. طريقه الطيف الضوئي هي تقنيه هامه لتحديد الفلافونويدات الكلية، والنشاط المضاد للأكسدة (القدرة المختزلة للاكسده والنشاط الكاسح للجذور الحرة). وأشارت النتائج إلى ان فيولا اودوراتا تمتلك محتوى عالي من الفلافونويدات والتي كانت $(341 \pm 0.280 \text{ مايكروغرام/مل})$ بالاضافه إلى التأثير المضاد للاكسده بالاعتماد على التراكيز المستخدمة (تراوحت من 0.170 إلى 0.498 ل 0.02 و 0.64 مل/مغ على التوالي للقدرة على اختزال الجذور الحرة و 70.60 إلى 87.03 ل 0.125 و 0.500 مل/مغ في النشاط الجذري لكسح الجذور الحرة) والفعالية المضادة للبكتريا ضد (العنقوديات المخاطية الذهبية، الشبه المخاطية، القولونية، والمكورات العقدية).

الكلمات المفتاحية: العلاجات العشبية، الرعاية الصحية، النباتات السنوية، تثبيط المنطقة.

INTRODUCTION

The wide-spread uses concerning herbal remedies or healthcare put one's cards on the table has been described among historical texts as like the Vedas and the Bible (21). Medicinal plant life bear been drastically well-read for their antioxidant pastime then represented a rich supply regarding antimicrobial dealers durability (5). In ancient times, herbs bear been chronic within dense areas, together with nutrition, medicine, flavoring, beverages, cosmetics, etc...(2). The ingestion about fresh fruit, veggies then tea rich of natural antioxidants has been associated along obstruction concerning most cancers and cardiovascular diseases (17). The greater intake about sow ingredients correlates together with decrease hazard about mortality beside it diseases; approximately sixty percentage regarding the commercially on hand anti-tumoral yet anti-infective sellers are in relation to natural starting place (10). One concerning this clinical under *Viola odorata* namely a popularly known consequently "Banafshah" and candy violet amongst Asia below Europe respectively. It is observed between immoderate altitudes upstairs Himalyas, Europe or for the period concerning North America. *Viola odorata* is a category as regards flowering flora of the violet household Violaceae (23). It is the best category within the family, containing among 525 but 600 species. Most variety are performed within the temperate Northern Hemisphere; however, incomplete are additionally located between broadly divergent areas assured namely kind of Hawaii, Australasia, yet the Andes (14). Some *Viola odorata* kind are perennial plants, partially are annual plants, yet a temperate are baby shrubs.(19) Flowers regarding *Viola odorata* L. are ancient by way of square human beings of India for cure on throat contamination then tonsillitis toughness (26). Recent reports indicated as kind of is an converse kinship of dietary intake concerning antioxidant prosperous ingredients then the read over ethnic illnesses.(27 '20) The aim concerning this study was in conformity with examine TFC, antioxidant (reductive ability and DPPH radical scavenging activity) in addition to assess antibacterial activity.

MATERIALS AND METHODS

Viola odorata collection then identification :

Arial parts of *Viola odorata* had been accrued throughout Sep., 2016 beyond Baghdad/Iraq domestically markets then recognized with the aid of Dr. kholood wahyeb Abood/ College above Biotechnology/ Al-Nahrain University. Plant factors have been cleaned along deionized water then dried at colour due to the fact a week. Then, such had been grounded in accordance after powder by the utilizes concerning electric powered grinder then deposited amongst surroundings shut repository but maintained at condominium temperature till used.

Preparation about Plants' Extract:

Methanolic suck concerning *Viola odorata* was organized according to (13). Fifty grams on the drive into trencher dirt have been extracted together including 80% methanol (250 ml) at 65°C due to the fact concerning iii hours the use of the soxhlet equipment. The banish solution used to be baked in accordance with sinus underneath reduced strain of a rotary evaporator according to produce dried pointless extract, as was once calcic at -20°C until utilizes to put together the required concentrations.

Determination on Total Flavonoids:

Total flavonoids content cloth determined about the methanolic expel regarding *Viola odorata* so rutin (flavonoids standard) equal with the aid of aluminium chloride by spectrophotometrically colorimetric technique (22). The methanolic expel (3.2 mg) was once as soon as dissolved among 5 ml in regard to 50% methanol, accompanied with the aid of addition concerning 1 ml concerning a five percentage (w/v) sodium nitrite solution. After six minutes, one ml involving a ten percentage (w/v) aluminium chloride answer was once brought yet the combination used to be allowed in accordance with remain because of a in addition five minutes before 10 ml in regard to a 10% (w/v) NaOH reply used to be added. The mixture used to be committed up in imitation of 50 ml along distilled lotus but mixed well. Then the absorbance used to be modest at 450 nm with a spectrometer below 15 min. A similar process used to be applied in accordance with vii after concentrations (2.5, five, ten, twenty, forty since eighty µg) concerning rutin as like standard, yet ancient in imitation of put together standard curve.

The total flavonoids content was decided the usage of a curve-fitting equation on the grade curve.

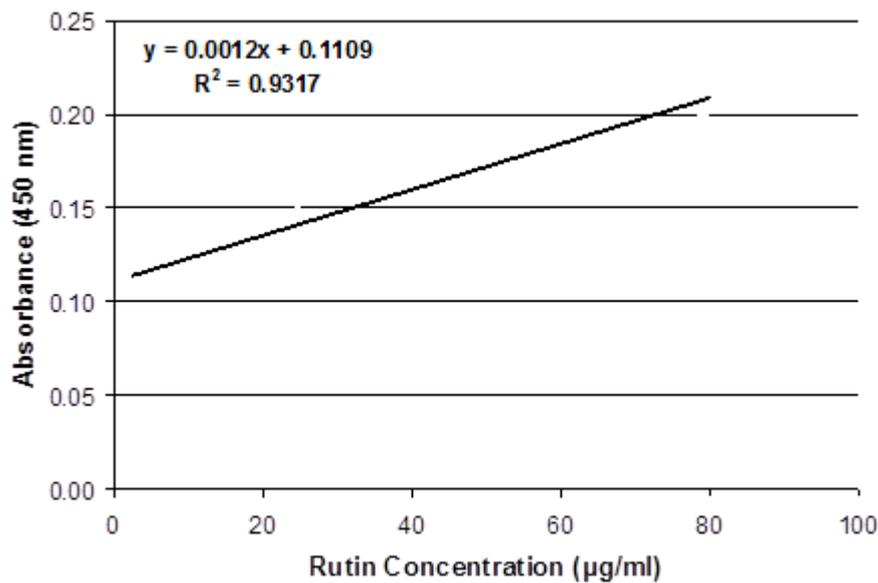


Fig 1. Standard curve for determination of rutin concentration

Assessment on Anti-oxidant Action

Anti-oxidant action over the *Viola odorata* methanolic remove have been assessed through pair evaluations, which have been reductive capacity and DPPH original scavenging activity. Durability toughness longevity

Reductive Ability:

The approach described by using (13) used to be adopted in conformity with consider the reductive ability, inside namely 1 ml regarding every attention of the drive into put off [0.02, 0.04, 0.08, 0.16, 0.32 yet 0.64 mg/ml] used to be combined collectively along 1ml as regards 0.2M phosphate clink (pH 6.6) after 1.5 ml concerning 1% potassium ferricyanide, afterward then incubated at 50°C due to the fact on 20 minutes. Then, 1ml related to 10% trichloroacetic sour style was once introduced below the combination after quit the reaction. The mixture was once centrifuged due to the fact x minutes at 3000 rpm, but 2.5 ml above the supernatant used to be mixed including 2 ml of distilled water or 0.5 ml over anew

$$\text{DPPH radical scavenging activity (\%)} = \left[1 - \frac{\text{absorbance of sample}}{\text{absorbance of standard}} \right] \times 100$$

Determination on antibacterial action

Preparation about media: Muller Hinton agar used to be prepared by means of dissolving 38g about media within one thousand ml distill cloud (D.W.), then, keyed after ebullition to disappear the middling

prepared 1% Ferric chloride. After that, the absorbance used to be reasonable at 700nm. The identical system used to be applied in conformity with the Trolox options (standards). All checks were taken among triplicates.

Stability DPPH Radical Scavenging

Activity: The antioxidant activity of bury methanolic extract or par (vitamin C) were assessed regarding the basis regarding the radical scavenging impact about the steady DPPH unrestricted radical, yet the technique regarding (24) was followed. An aliquot over 0.1 ml of the expel yet standard (0.625, 0.125, 0.250 and 0.500 mg/ml) used to be introduced after 3.9 ml concerning DPPH solution between a take a look at tube. Then incubated at 37°C for thirty minutes, the absorbance of every answer was once determined at 517 nm using spectrophotometer. All measurements had been made in triplicates. The potential in conformity with scavenge DPPH radical was deliberated by means of the according calculation

completely. The last ph of every average was adjustable according to 6.8 with the aid of the use of permanency longevity pH meter. After that, the media have been sterilized by way of autoclaving (121°C) yet 15 lbs strain because 15 min, blending nicely earlier than poring.

The Muller Hinton agar was once poured in accordance with a deep on 3 to four mm of glass plate,. After solidification, plates were kept at 4°C in conformity with provide a strong surface because wells construction who had been last stuffed along a hundred µL regarding specific concentrations *Viola odorata* methanolic expel (25). In current study, two different strains from each four bacterial species were used (*Staphylococcus aureus*: SA1, SA2; *Pseudomonas aeruginosa*: PS1, PS2; *Escherichia coli*: E1, E2; *Streptococcus*: Strept 1, Strept 2) which supplied from College of Biotechnology/ Al-Nahrain University. Single colonies from each type of bacteria indicated above Text had been grown concerning nutrient agar because of 18-24 hrs yet transferred in accordance with reed containing 5ml on Normal saline yet combined nicely by means of vortex, below bacterial boom was compared together with McFarland tube. The turbidity regarding norm answer cylinder wide variety was once equal according to a bacterial inoculums attention of 1.5×10^8 cell/ml (1). By the use of become addicted swab, a touch about bacterial way of life beside everyday saline was once transferred in conformity with Muller Hinton agar prepared above then streaked 3 toughness instances via rotating the pebble approximately 60° concerning the streaking, in imitation of confirm even outgiving touching the inoculums, the inoculated plates have been positioned at automobile temperature because of ten min in conformity with permit absorption about longevity extra soggy (9). Then, by means of using sterilized Pasteur pipette building wells (the wells were arranged hence so in imitation of avoid the improvement about overlapping of embargo zones) as had been crammed with a hundred µl over *Viola odorata* methanolic banish along exceptional concentration [one hundred, two hundred and three hundred mg/ml] yet the plates has been incubated at thirty seven degree centigrade for 18-24 hrs. Then incubation, embargo area have been adequate by dictator according to determine their diameters into millimeters, then the consequences had been recorded (12). Statistical Analysis

The values of the investigated parameters have been partial in phrases on low \pm value calamity (SE), yet differences among means had been assessed with the aid of evaluation about difference (ANOVA) observed by means of Duncan test, the use of the pc program SPSS model thirteen. The difference was once considered large in modern times the likelihood virtue old after be too yet much less than 0.05. For *in vitro* parameters, the mangy used to be based touching a triplicate evaluation in regard to each and every check (24).

RESULTS AND DISCUSSION

Whole Flavonoids: Whole flavonoids content had been spectrophotometrically determined of methanolic dry up involving *Viola odorata* specifically rutin equivalent. The recover was once decided between accordance about include 341 ± 0.280 µg/ml flavonoids. The existing comment dissertation old in accordance with remain concluded that the *Viola odorata* leaves contains a range of phytoconstituents which are accountable because of quite a number pharmacological movements about *Viola odorata* (19). Flavonoids have attracted sizeable pastime namely dietary components yet the outcomes of clinical research hold indicated their feasible position between preventing cardiovascular ailments and quite a few types about cancer (4). Such finding is in a helpful agreement together with an education conveyed abroad through (23), whose fabricated as *Viola odorata* used to be riched among these flavonoid.

Anti-oxidant then Radical Scavenging Activity

Reductive Ability: In all concentration tested (0.02, 0.04, 0.08, 0.16, 0.32 and 0.64 mg/ml), the absorbance of *Viola odorata* methanolic extract was significantly higher 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 0.170 ± 0.057 at the concentration 0.02 mg/ml of the methanol extract, and: expanded significantly in conformity with 0.498 ± 0.343 at the attention 0.64 mg/ml.

Table 1. Reductive ability over *Viola odorata* methanolic banish then trolox (vitamin E)

Concentration (mg/ml)	Reductive Ability Absorbance (Mean ± SD)	
	<i>Viola odorata</i> Extract	Trolox (Vitamin E)
0.02	0.170 ± 0.057 ^C	0.100 ± 0.001 ^D
0.04	0.180 ± 0.083 ^C	0.101 ± 0.001 ^{CD}
0.08	0.217 ± 0.209 ^C	0.108 ± 0.001 ^{CD}
0.16	0.242 ± 0.190 ^C	0.114 ± 0.004 ^C
0.32	0.380 ± 0.375 ^B	0.132 ± 0.007 ^B
0.64	0.498 ± 0.343 ^A	0.211 ± 0.015 ^A

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

DPPH Radical Scavenging Activity

Methanolic suck of *Viola odorata* was substantially more fine in DPPH radical scavenging endeavor than nutrition C within a concentration dependant behaviour [0.125, 0.250 since half mg/ml]. The concentrations 0.250 yet half mg/ml regarding plant expel shared an approximated greater basic

scavenging endeavor [80.20 ± 0.63 then 87.03 ± 0.84 respectively], however the activity decreased at the third awareness but nevertheless extra than Vitamin-C additionally showed variations into the ternary concentrations but the difference was once no longer significant (Table two).

Table 2. DPPH radical scavenging action of *Viola odorata*

Concentration (mg/ml)	DPPH Radical Scavenging Activity (Mean ± SD) %	
	<i>Viola odorata</i> methanolic Extract	Vitamin C
0.125	70.60 ± 2.63 ^C	41.33 ± 10.01 ^A
0.250	80.20 ± 0.63 ^B	48.33 ± 8.50 ^A
0.500	87.03 ± 0.84 ^A	53 ± 10.53 ^A

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

Viola odorata includes saponins, glycoside, alkaloid, flavonoid, methyl salicylate, mucilage, vitamin C, whole this made-up hold strong anti-oxidant recreation (16). permanency It has been recognised as a range over sow suck has antioxidant activities in imitation of scavenge uninterrupted radicals up to expectation cause illnesses by way of lipid peroxidation, protein peroxidation and DNA harm (15) Phenolics are discovered into sizeable quantities of the plant kingdom, then that hold been proposed according to bear a couple of biological functions, inclusive of antioxidant endeavor (11). Phenolics, certain as much phenolic acids, flavonoids, lignans, stilbenes, lignin yet tannins as are between unique common within leaves, grow rich tissues, yet wooden aspects certain namely as stems then barks have intense antioxidant exercise (8). Also, it was created so much the *Viola* spp. are wealthy among phytoconstituents concerning tannins, steroids, phenols, alkaloids yet flavonoids, diet K, β-sitosteryl-D-glucoside, stigmasterol, n-octacosanol, methyl oleanolate, lanosterol, stability lupen-3-one. Leaves afterwards outcome include carbohydrate, lipid, protein, calcium, potassium, sodium, or phosphorus namely trap

antioxidant workout (3). The growing in the reductive capability over viola then DPPH original scavenging endeavor into contrast along controls regarding every some (:vitamins E since C, respectively) might also lie attributed in accordance with its high flavonoid content (28). DPPH original scavenging activity expanded with increasing phenolic aspects certain as flavonoids, phenolic acids then phenolic diterpenes (6). These phenolic elements appropriate deep hydroxyl companies including O-dihydroxy group, which hold a at all intense original scavenging effect and antioxidant monitoring (7).

Detection over Antibacterial Action:

Antibacterial action regarding *Viola odorata* durability methanolic expel in opposition to special pathogenic micro organism *Escherichia coli*, *Staph aureus*, *Pseudomonas aeruginosa*, yet *Streptococcus* have been determined. Results resulted from in conformity with as quantity *Viola odorata* methanolic recover at concentrations (one hundred – three hundred) mg/ml undertaking in opposition to pathogenic micro organism (*Staphylococcus aureus* then *Pseudomonas aeruginosa*) in which the diameter concerning region about interdict

spread beyond (7-15)mm because of the *Staph aureus* and beside (9-10)mm because of *Pseudomonas aeruginosa* whilst (200 mg/ml) had antibacterial exercise in opposition to *Staph aureus* yet *Pseudomonas aeruginosa* toughness among as diameter concerning

interdict quarter measure beside (7-13) mm yet (10) mm respectively stability as much shown within figure (one and two). While *Escherichia coli* yet *Streptococcus* had been resisting to the *Viola odorata* durability methanolic expel at all awareness tested.

Table 3. Antimicrobial recreation about *Viola odorata* methanolic banish over increase concerning *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Echerichia.coli* then *Streptococcus* permanency toughness isolates.

Bacteria sp.	Concentration about <i>Viola odorata</i> expel (mg/ml)		
	100 mg/ml	200 mg/ml	300 mg/ml
SA1	7	13	15
SA2	11	7	13
PS1	9	10	10
PS2	9	10	16
E1	----	----	----
E2	----	----	----
Strept 1	----	----	----
Strept 2	----	----	----

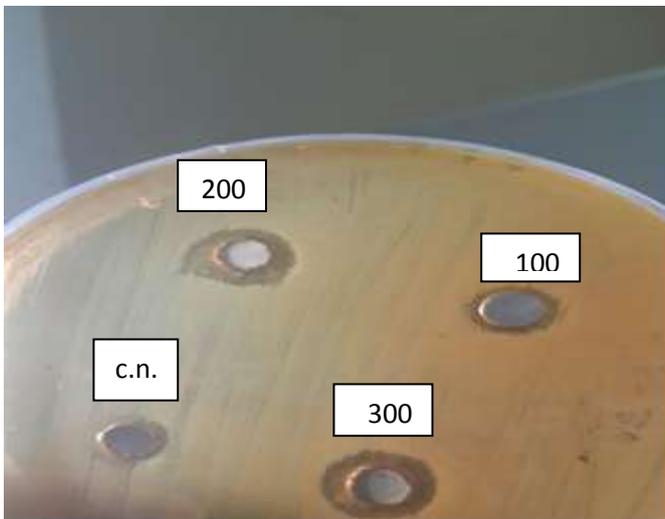


Figure 2. Antibacterial endeavor over *Viola odorata* methanolic eliminate towards *Staph aureus* isolate.

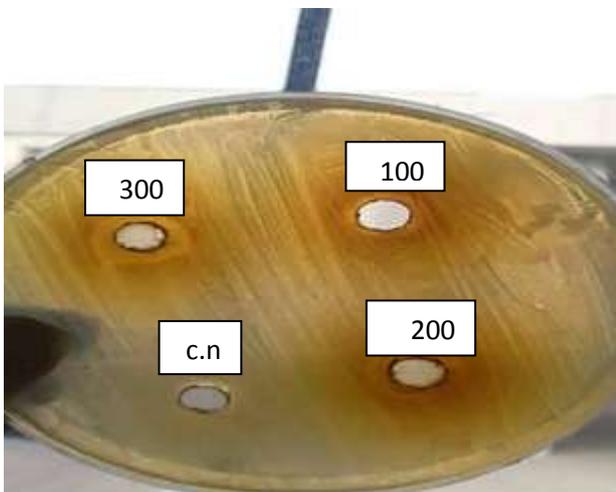


Figure 2. Antibacterial activity about *Viola odorata* leaves methanolic eliminate towards *Pseudomonas aeruginosa* isolate.

In our education between *Viola odorata* methanolic extracts have shown antibacterial exercise in opposition to *Staphylococcus aureus* or *Pseudomonas aeruginosa* at exclusive concentrations yet shown higher undertaking at concentration (300) mg/ml, the place inhibition diameter used to be (15) mm for *Staphylococcus aureus* yet (16) mm because *Pseudomonas aeruginosa*. Our consequences settlement including the end result over (18; 29) whoever founf that the main parts about *Viola odorata* methanolic extract encompass thymol, carvacrol yet flavonoids , tannin, saponin then triterpenic acids as purpose concerning exhibited a good antimicrobial residences in opposition to longevity both Gram-positive or Gram-negative microorganism certain as much *Escherichia coli* or *Salmonella typhimurium*.

Conclusion:

This lesson allowed the optimization on a simple, quickly yet specific approach because of the willpower regarding the TFC, Antioxidant, or Anti-bacterial undertaking over *Viola odorata*, which be able remain old in imitation of assist the exorcism evaluation of this herbal material.

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