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Short Communication

# An in vivo Evaluation of the Analgesic Activity of Date (*Phoenix dactylifera L.*) Fruit Tablets along with the Olive (*Olea europaea*) Leaves Extract

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## Abstract

**Background and Aim:** This paper reports, for the first time, the in vivo analgesic activity (AnA) of date (*Phoenix dactylifera* L., variety *Mech-Degla*) fruit tablets along with 3 % (w/w) lyophilized aqueous extract of olive (*Olea europaea*) leaves (DT-OLE).

**Materials and Methods:** For both the date fruit powder (DFP) and olive leaves extract (OLE), flavonoids, alkaloids, tannins, saponins and anthocyanins were qualitatively identified. In the case of OLE, the qualitative screening of phenolic compounds was also carried out using HPLC. The AnA (in%) was evaluated through the acetic acid writhing test on 30 NMRI mice.

**Results:** The DT-OLE at a dose of 200 mg / kg, bw showed an interesting AnA value (42%), which is equivalent to approximately 0.44 times of that (96%) of paracetamol (taken as reference), with the DFP displaying the lowest AnA (4%). Thus, the analgesic properties of DT-OLE might be attributed to OLE, and the DFP with the role of the taste masking agent.

**Conclusion:** The AnA of DT-OLE was demonstrated. However, further studies are required. In all cases, considering their composition, investigated tablets might be classified as healthy dried infusions, healthy natural candies or functional food.

**Keywords:** Analgesic activity, Date fruit, Olive leaves, Tablet, Mice

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## Introduction

In some circumstances, due to the undesirable side effects of drugs, patients have little choice but to seek alternative treatment modalities, which has led to the latter's ever-increasing popularity in recent years (1).

The date (Phoenix dactylifera L.) fruit, which is well known for its sweet taste, can also be considered as functional food due to its richness in bioactive compounds (2). Nevertheless, precise remedial effects

of date palm have not been completely examined (3). Olive (*Olea europaea*) leaves have been used since ancient times in traditional Mediterranean medicine. They are known in most countries as a traditional remedy for hypertension, diabetes, rheumatic diseases, etc. (4). More recently, Moghaddasi *et al*. (5) reported the possible beneficial impacts of the olive leaves ethanolic extract in managing the cerebral hypoperfusion-induced cardiac abnormality in rats. Olive leaves are also used as ingredients in various food, cosmetic, nutraceutical, and pharmaceutical formulations (6). Olive leaves are now used in the European Pharmacopoeia (7)

There are a number of studies dedicated to the AnA of date fruits (3, 8)) and olive leaves (9-11). Nevertheless, to the best of our knowledge, no work has been dedicated on the AnA of food supplements and phytopharmaceuticals combining date fruits and olive leaves. The latter are widely known to be rich in oleuropein, and their most abundant bioactive compound is characterized by a very pronounced bitterness. This bitterness in turn affects their acceptance by consumers. In this respect, the use of date fruits as a universal carrier for plant extracts in general, and olive leaves in particular, has been suggested (12). In the United States of America and Europe OLE has been recognized as a dietary supplement (13).

This paper reports the analgesic activity (AnA) in vivo (mice) of tablets (DT-OLE) from date (*Phoenix dactylifera* L., variety *Mech-Degla*) fruit powder (DFP) along with the olive (*Olea europaea*) leaves lyophilized aqueous extract (OLE) at rate of 3% (w/w).

## Materials and Methods

### Plant Material, Phytochemical Screening and Tableting

The date fruits (*Phoenix dactylifera* L., variety *Mech-Degla*) used in this work belong to the so-called common variety groups. They are characterized by a dry or semi-dry consistency and a low market value. The fruits were bought in Tolga (the southern region of Biskra, in the Algerian Sahara).

The olive (*Olea europaea*, variety *Chemlal*) leaves were harvested in the region of Boumerdes (Eastern Algiers). The preparation of the DFP and OLE was carried out according to the procedures described by Iguergaziz *et al*. (12). They are briefly recalled here. Regarding the DFP, the whole date fruits were dried at 40˚C in a ventilated oven (type GENLAB OVEN) to constant weight. They were then crushed and sieved to obtain a particle size powder ~ 400µm. The DFP was then stored at 4 °C in airtight containers protected from light, until use.

The OLE was obtained by lyophilization of an aqueous extract previously prepared from olive leaves dried at 40˚C for 24 hours. OLE was kept out of light in airtight containers at 4 ° C, until use. For both OLE and DFP, flavonoids, alkaloids, tannins, saponins and anthocyanins were identified according to qualitative chemistry methods described by Iqbal *et al*. (14).

In the case of OLE, the qualitative screening of phenolic compounds was also carried using HPLC according to the method described by Hayes *et al*. (15). The HPLC was performed on the Agilent technologies 1100 series platform, equipped with a UV-Visible detector. The column used was C18 (5 µm, 4.6 × 150 mm). Elution was performed at 25 °C in gradient mode using a binary solvent mixture comprised of water acidified with 1% acetic acid (solvent A) and 100% methanol (solvent B). The flow of the mobile phase was 1 mL/min and the injection volume of each sample was 20µL. Phenolic monomers were detected at 280 nm. The peaks of the phenolic compounds (oleuropein, rutin, quercetin and caffeic acid) were identified via making a comparison between their retention time with those of standards analyzed in the same conditions.

The DT-OLEs were prepared at the Research and Development Center (CRD, SAIDAL, GDC, Algiers) by direct compression using an eccentric press (type ED. Frogerais, OA 307). The proportion (3%, w/w) of OLE in the tablets was chosen taking into account consumer preference highlighted by a taste test (12).

### Animals

Thirty NMRI breed mice (20 ± 2 g each) were used in the present study. These animals were provided by the pet shop of the Research and Development Center, Saidal, Algiers. They were reared under standard environmental conditions corresponding to a temperature of 24°C and a cycle of light 12h / 24h. The mice were fasted 17 h before the experiment. They were handled according to guidelines of the EU Directive

2010/63/EU for animal experiments.

**AnA Evaluation**

The AnA evaluation was carried out using the acetic acid writhing test which is known to be among the most common tests in evaluating the analgesic activity of non-narcotic analgesics (16). The method reported by Koster *et al*. (17) was applied. The mice were divided into five groups (G) of six mice. One group received paracetamol, whereas other groups received processed plant products (diets) to be tested, namely DFP, OLE and DT-OLE. Paracetamol and plant products were suspended in distilled water and then administered to the mice by gavage at a rate of 0.5 ml/mouse (Table 1). The diets were administered in form of suspensions in 15 mL distilled water. It might be worthwhile to remark that the pure OLE dose of 6 mg/kg (bw) was deduced from the rate (3% ww) used in DT-OLE3 (18).

After 30 min, 1% acetic acid was injected intraperitoneally at a rate of 0.1 ml / mouse. Ten min after the injection of acetic acid, the number of writhing movements in the form of contractions of the abdominal muscle was counted for each mouse for 10 min.

The AnA, expressed as percentage inhibition of writhes, was calculated according to the following equation:

$$AnA\ (\%) = 100.\ (1\text{-}C_t/C_c)$$

where $C_t$ is the mean of writhes observed in the treated group (G3, G4 or G5), and $C_c$ is the mean of writhes observed in group G1 (negative control).

**Statistics**

The results are given as mean ± standard deviation. The Tukey one-way analysis of the variance (ANOVA) was performed using the XLSTAT software (2014). The difference was considered significant at $p < 0.05$.

All the animal experiments were observed according to the EU Directive 2010/63/EU for animal experiments.

**Table 1:** Animal Groups and Diets to be Tested for AnA.

| Groups | Diet to be tested |
|---|---|
| **G1** | Distilled water (0.5 mL/animal) |
| **G2** | Paracetamol (200 mg/kg, bw) |
| **G3** | DFP (194 mg/kg, bw) |
| **G4** | OLE (6 mg/kg, bw) |
| **G5** | DT-OLE (200 mg/kg, bw) |

## Results and Discussion

**Chemical screening**

The qualitative phytochemical screening has been given in Table 2.

Concerning DFP, the results were in line with the scientific literature. For example, Al-daihan and Bhat (19) revealed the presence of flavonoids, tannins, alkaloids and saponins in the Mosaifah cultivar grown in Saudi Arabia. In the same way, on more than 50 varieties of dates considered in a bibliographic research, Al-Shwyeh (20) also only mentioned this cultivar as a source of alkaloids and certain black varieties as sources of anthocyanins. With regard to OLE, flavonoids, alkaloids, saponins, tannins were cited to be contained in the aqueous extract of olive leaves (21). The absence of anthocyanins in OLE was to be expected since this group of polyphenols is mainly synthesized in colored fruits and vegetables. It was already reported that flavonoids, tannins and alkaloids from medicinal plants could exhibit analgesic and anti-inflammatory properties in animal models (22, 23). Moreover, the total absence of saponins and anthocyanins in the two powders should be noted.

The HPLC- phenolic profile of OLE has been shown in Figure 1. As it can be seen, the relative abundance of different compounds (with their respective retention times) can be estimated in the following decreasing order: oleuropein>rutin>quarcetin≈caffeic acid. These

**Table 2:** Results of the Qualitative Phytochemical Screening of DFP and OLE.

| Component | DFP | OLE |
|---|---|---|
| Flavonoids | + | + |
| Alkaloids | - | + |
| Tannins | + | + |
| Sapononins | - | - |
| Anthocyanins | - | - |

DFP: date fruit powder, OLE: lyophilized aqueous olive leaves extract, +: presence of phytochemical component, and -: absence of phytochemical component.

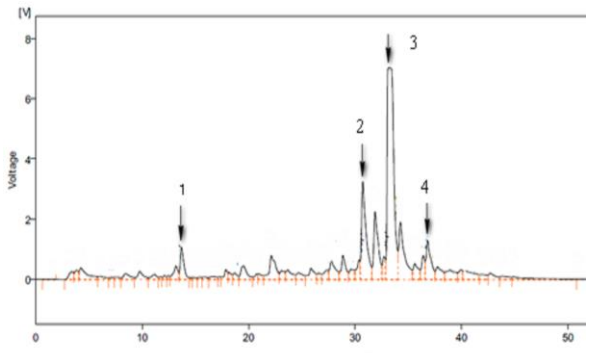


**Figure 1.** HPLC- polyphenolic compounds of olive leaves extract (OLE): caffeic acid (1: 13.3 min), rutin (2: 30.5 min), oleuropein (3: 33.2 min), and quercetin (4: 36.8 min).

findings are in concordance with those reported by numerous authors who underlined the predominance of the oleuropein in OLE (13, 22, 24). Moreover, it is known that polyphenols are able to train a synergistic interaction in terms of biological activities.

**AnA results**

The obtained AnA values have been illustrated in Figure 2.

It should be emphasized that the AnA value of AnA should vary between 0 (when Ct =Cc, i.e. in the case of G1) and 100% (when Ct = 0). The same lowercase letters above the columns indicate no significant difference ($p>0.05$) between the corresponding AnA values. See Table 1 for the identification of rat groups (G1, G2, G3, G4 and G5). From this figure, the AnA of the G2 group (positive control) (96%) was the highest ($p<0.05$), followed by that (~ 43 %) of the G4 (fed with OLE) and G5 (fed with DT-OLE) groups. Moreover, no significant difference was observed between AnA values for these last two groups ($p>0.05$). The obtained results were consistent with those reported by Esmaeili-Mahani *et al*. (25) regarding ethanolic extracts of olive leaves at doses of 200 mg/kg. These authors reported pain inhibition rates of approximately 42% and 62% depending on pain assessment mode. Furthermore, the AnA values reported here are higher than those (about 35 %) found by Zihad *et al*. (26) for Bangladeshi medicinal plant *Chrysopogon aciculatus* (at the dose of 500 mg/kg b.w. orally) and Jo *et al*. (27) for Korean medicinal plant *Aucklandia lappa* (sun-dried root at dose 150 mg/kg)

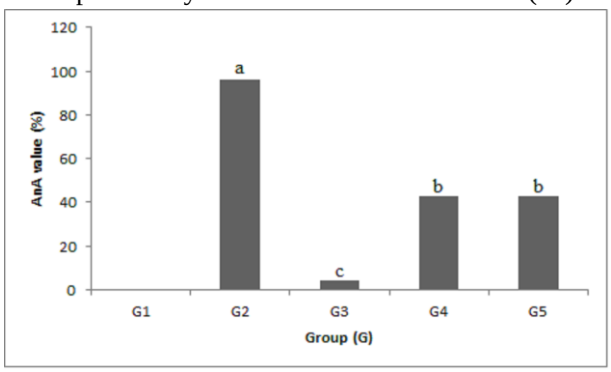


**Figure 2.** Analgesic Activity (AnA) the Versus Mice Groups Differently Treated.

The AnA value (~4%) of the G3 group (fed with DFP) was 10 times less than that of OLE. The AnA of the G3 group with the G4 and G5 groups were compared. It was revealed that the DT-OLE3 AnA could only be attributed to the OLE as the minor component, and DFP served as the taste masking agent. Nevertheless, other authors have reported analgesic and ant-inflammatory activities of the date fruit (28). We believe that the analgesic activity of OLE could be attributed mainly to the combination of the effects of polyphenols and alkaloids which are known for their painkilling properties (25, 29, 30). Bahmani *et al*. (31) have pointed out that the analgesic effects of flavonoids and alkaloids could be explained by the inhibition of prostaglandin synthesis. At last, it seems there is an additive analgesic effect between DFP and OLE. As it has been indicated in figure 1, the observed AnA for DFT=3.8 % and for OLE was 39.5 %. It should theoretically be as follows: $AnA_{DT\text{-}OLE} = (AnA_{DFP})_{observed} + (AnA_{OLE})_{observed} = 3.8 + 39.5 = 43.3$ %

The real value (43 %) of the AnA observed for DT-OLE (from Figure 1) is very close to the expected theoretical value (43.3 %), thus proving the additive analgesic effect between DFP and OLE.

## Conclusion

The AnA of DT-OLE was demonstrated. However, further investigations are recommended. In all cases, considering their composition, the investigated tablets might be classified as healthy dried infusions, healthy natural candies, or functional food.

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## Conflict of Interest

The authors declare that they have no conflict of interest.

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## References

1. Jahromi B, Pirvulescu I, Candido KD, Knezevic NN. Herbal medicine for pain management: Efficacy and drug interactions. Pharmaceutics. 2021;13(2):251.
2. Alvi T, Khan MKI, Maan AA, Razzaq ZU. Date fruit as a promising source of functional carbohydrates and bioactive compounds: A review on its nutraceutical potential. Journal of Food Biochemistry. 2022;46(9).
3. Al-Okbi, S.Y. Date Palm as Source of Nutraceuticals for Health Promotion: a Review. Current nutrition reports. 2022;11:574-91.
4. Lee OH, Lee BY, Lee J, Lee H-B, Son J-Y, Park C-S, et al. Assessment of phenolics-enriched extract and fractions of olive leaves and their antioxidant activities. Bioresource Technology. 2009;100(23):6107-13.
5. Moghaddasi M, Taati M, Noroozi H, Lirgeshasi SM, Pourkhodadad S, Bahram Delfan B, et al. The effects of olive leaf extract administration on the cerebral hypoperfusion-induced electrophysiological alterations in rat heart. Herbal Medicines Journal. 2017;2(3):105-13.
6. Erbay Z, Icier F. A review of thin layer drying of foods: Theory, modeling, and experimental results. Critical Reviews in Food Science and Nutrition. 2010;50(5):441-64.
7. Scheffler A, Rauwald HW, Kampa B, Mann U, Mohr FW, Dhein S. Olea europaea leaf extract exerts L-type Ca(2+) channel antagonistic effects. Journal of Ethnopharmacology. 2008;120(2):233-40.
8. Shabani M, Zangiabadi N, Asadi-Shekaari M. Evidence for positive effects of date extract that attenuates thermal hyperalgesia in a diabetic rat model of neuropathic pain. Neuroscience and Medicine. 2013;4:16-22.
9. Laaboudi W, Ghanam J, Aissam H, Merzouk Mi, Benlemlih M. Anti-inflammatory and analgesic activities of olive tree extract. International Journal of Pharmacy and Pharmaceutical Sciences. 2016;8(7):414-19.
10. Ghazi AM, Al-Baghdadi RJT. Evaluation of the antinociceptive and antipyretic effects of the Iraqi olive leaf extract in murine animals. Journal of Pharmaceutical Sciences and Research. 2018;10(11):2916-20.
11. Ullah S, Iqbal J, Khan AU, Aman T, Razaque G. Neuro and analgesic activities of olive leaf methanolic crude extract. Indo American Journal of Pharmaceutical Sciences. 2018;5(3):1814-21.
12. Iguergaziz N, Benamara S, Boukhiar A, Djallouli F-Z, Guebrili A, Angar N-E, Bitam A. Release characteristics of paracetamol and oleuropein from Mech-Degla date fruit tablets enriched and non-enriched with freeze-dried olive leaf extract. Chemical Engineering Communications. 2019; 206(4):524-34.
13. Du GH. Natural small molecule drugs from plants, Springer, 2018, Singapore.
14. Iqbal E, Abu Salim K, Lim, Linda BL. Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of Goniothalamus velutinus (Airy Shaw) from Brunei Darussalam. Journal of King Saud University - Science. 2015;27(3):224-32.
15. Hayes JE, Allen P, Brunton N, O'Grady MN, Kerry JP. Phenolic composition and in vitro antioxidant capacity of four commercial phytochemical products: Olive leaf extract (Olea europaea L.), lutein, sesamol and ellagic acid. Food Chemistry. 2011;126(3):948-55.
16. Slyusarenko VS, Koklin IS, Kovalenko SM, Chuev VP, Shabalin AA, Gureev VV, et al. Study of analgesic activity and effects of new dipharmacophores – nebracetam and cyclooxygenase-2 inhibitors derivatives on the cognitive abilities of rats. Research Results in Pharmacology. 2021;7(4):71–9.
17. Koster R, Anderson M, De Beer J. Acetic acid for analgesic screening. Fed Proc.1959;8:412-17.
18. Clewell AE, Béres E, Vértesi A, Glavits R, Hirka G, Endres JR, et al. A comprehensive toxicological safety assessment of an extract of Olea Europaea L. leaves (Bonolive™). International Journal of Toxicology. 2016;35:208-21.
19. Al-daihan S, Bhat RS. Antibacterial activities of extracts of leaf, fruit, seed and bark of Phoenix dactylifera. African Journal Biotechnology. 2012;11(42):10021-5.
20. Al-Shwyeh HA. Date palm (Phoenix dactylifera L.) Fruit as potential antioxidant and antimicrobial agents. Journal of Pharmacy and Bioallied Sciences. 2019;11(1):1-11.
21. Khan H, Ahmad W, Hussain I. Imran M, Afridi MS, Ullah S. Phytochemical composition, antioxidant and antimicrobial activities of leaves of Olea europaea wild variety. Journal of Food Measurement. 2020;14:640-8.
22. Shojaii A, Motaghinejad M, Norouzi S, Motevalian M. Evaluation of anti-inflammatory and analgesic activity of the extract and fractions of Astragalus hamosus in animal models. Iranian Journal of Pharmaceutical Research. 2015;14(1):263-9.
23. Hayfaa AA-S, Sahar AAMA-S, Awatif MA-S. Evaluation of analgesic activity and toxicity of alkaloids in Myristica fragrans seeds in mice. Journal of Pain Reserach. 2013;6:611- 5.
24. Xie P-J, Huang L-X, Zhang C-H, Zhang Y-L. Phenolic compositions, and antioxidant performance of olive leaf and fruit (Olea europaea L.) extracts and their structure–activity relationships. Journal of Functional Foods. 2015;16,460–71.
25. Esmaeili-Mahani S, Rezaeezadeh-Roukerd M, Esmaeilpour K, Abbasnejad M, Rasoulian B, Sheibani V, Kaeidi A, Hajializadeh Z. Olive (Olea europaea L.) leaf extract elicits antinociceptive activity, potentiates morphine analgesia and suppresses morphine hyperalgesia in rats. Journal of Ethnopharmacology. 2010;132(1):200-5.
26. Zihad SMNK, Bhowmick N, Uddin SJ, Sifat N, Rahman MS, Rouf R, Islam MT, et al. Analgesic activity, chemical profiling and computational study on Chrysopogon aciculatus. Frontiers in Pharmacology. 2018;9.
27. Jo H-G, Lee G-Y, Baek CY, Song HS, Lee D. Analgesic and anti-inflammatory effects of Aucklandia lappa root extracts on acetic acid-induced writhing in mice and monosodium iodoacetate-induced osteoarthritis in rats. Plants. 2021;10(42).
28. Kehili HE, Zerizer S, Beladjila KA, Kabouche Z. Anti-inflammatory effect of Algerian date fruit (Phoenix dactylifera). Food and Agricultural Immunology. 2016;27(6):820-9.
29. Faujdar S, Sharma S, Sati B, Pathak AK, Paliwal SK. Comparative analysis of analgesic and anti-inflammatory activity of bark and leaves of Acacia ferruginea DC. Beni-Suef University Journal of Basic and Applied Sciences. 2016;5(1):70-8.
30. Ganeshpurkara A, Saluja AK. The pharmacological potential of rutin. Review. Saudi Pharmaceutical Journal. 2017;25(2):149-64.
31. Bahmani M, Shirzad H, Majlesi M, Shahinfard N. A review study on analgesic applications of Iranian medicinal plants. Asian Pacific Journal of Tropical Medicine. 2014;7(1):43-53.

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