Ethnomedicinal Uses and Antimitotic, Analgesic and Anti-inflammatory Effects of the Aqueous Leaf Extract of *Rubus fruticosus* L.

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Abstract. *Rubus fruticosus* is widely used in traditional medicine for the treatment of pain, inflammation, infections, and metabolic disorders. This study evaluated the biological activities of its aqueous extract using *Allium cepa* root assay, carrageenan-induced paw edema, and tail flick analgesic models. In the *Allium cepa* assay, the extract showed a dose dependent reduction in mitotic index compared to the distilled water control, while colchicine produced a strong positive inhibitory effect. The highest concentration (12 mg/mL) exhibited the greatest antiproliferative activity, approaching that of colchicine at later time points. In the anti-inflammatory model, the extract demonstrated significant dose-dependent inhibition of carrageenan-induced paw edema over 6 hours, with the 500 mg/kg dose showing higher activity than 250 mg/kg, though less than indomethacin. In the tail flick test, the aqueous extract produced significant analgesic effects, particularly at 250 mg/kg, with maximal latency observed between 45 and 60 minutes. However, its activity remained lower than that of paracetamol. Overall, the findings indicate that *R. fruticosus* possesses notable antiproliferative, anti-inflammatory, and analgesic properties, supporting its traditional medicinal use. The observed effects may be attributed to bioactive phytochemicals such as flavonoids and polyphenols. Further studies are required to isolate active compounds and clarify mechanisms of action.

1 Introduction

Traditional medicine refers to any form of healthcare that is rooted in ancestral and cultural practices, distinct from scientific medicine, and is primarily passed down through oral tradition within communities of various cultures [1]. In many civilizations across the world, medicinal plants have long been used to cure a wide range of illnesses. They are the foundation of both traditional and modern medicine [2]. Due to their low cost and ease of access, are a fundamental part of healthcare in many regions [3].

At present, numerous medicinal plants are employed in the management of inflammation and for their analgesic properties [4][5].

For ages, *R. fruticosus* has been utilized in traditional medicine due to its hypoglycemic [6], anti-inflammatory, antimicrobial [7], and diuretic properties [8].

The aim of this study is to identify the ethnobotanical uses of *R. fruticosus* and to evaluate its antimitotic effect *in vitro* and its anti-inflammatory and analgesic effects *in vivo*.

2 Materials and Methods

2.1 Ethnobotanical Uses

To determine the ethnobotanical uses of *R. fruticosus,* an ethnobotanical survey is being conducted in the province of Al Hoceima following the protocol described by El-Mernissi *et al* [9]. Data collection was based on semi-structured interviews, conducted using a questionnaire with informants. These interviews focused on three main areas: the parts of the plant used, methods of preparation, and the dosages employed.

2.2 Plant extraction

Fresh leaves of *R. fruticosus* are left to dry in the shade for 5 days, then ground in a mill (Damay); the resulting leaf powder is used to prepare the extract, briefly: 50 mg of *R. fruticosus* leaf powder is macerated in 500 ml of distilled water for 24 hours. The macerate is then filtered through Wattman No. 1 filter paper; the water is removed using a rotary evaporator (Buchi); the resulting

extract is placed in glass jars and stored in the refrigerator at 4°C until further use.

2.3 Anti mitotic *in vitro*

To evaluate the antimitotic potential of the aqueous extract of *R. fruticosus*, we used a modified assay described by Raheel *et al*. [10] employing onion bulbs (Allium cepa). Root germination was induced by a 24-hour immersion in water, which allowed us to select onion bulbs with roots measuring between 1 and 2 cm for the study. The selected bulbs were then treated with different concentrations of the extract, namely 2 mg/mL, 6 mg/mL, and 10 mg/mL. This experimental setup also included positive controls using colchicine at a concentration of 2 mg/mL, as well as a negative control consisting of water.

After exposure periods of 24, 48, and 72 hours, the apical regions of the onion bulb roots were carefully excised (5 apices for each extract). The excised tissues were immersed in a 1 M HCl solution for 5 minutes, followed by gentle rinsing with distilled water, then fixed with aceto-carmin for chromosome staining. Subsequently, the samples were carefully crushed, allowing for the quantification of mitotic and total cells in 5 to 8 fields (400 to 500 cells) within each root tip.

This comprehensive microscopic analysis allowed for an accurate calculation of the mitotic index. Optical microscopy was performed using an Optika microscope (Italy), and images were captured with an Infinity 1 microscope camera. The mitotic index was calculated using the following formula:

$$Mitotic\ index = \frac{Number\ of\ dividing\ cells}{Total\ number\ of\ cells} * 100$$

2.4 Anti-inflammatory *in vivo*

The method developed by Charles A. Winter et al. was used for the rapid and sensitive evaluation of paw edema in rodents [11]. The Digital Water Plethysmometer consists of two interconnected tubes containing a conductive solution. When the animal's paw is immersed in the measuring chamber, displacement of the liquid alters the conductivity between two platinum electrodes. The difference between the volumes of the inflamed and non-inflamed paws corresponds to the edema volume (mL). Carrageenan was used as the inflammatory agent to induce paw edema.

Before treatment administration, the basal volume of the right hind paw was measured. The control, aspirin, and extract groups received the respective treatments orally by gavage 60 minutes prior to the subplantar injection of 0.1 mL of 1% carrageenan into the right hind paw. Paw volume was measured before carrageenan injection and again at 15 and 120 minutes after induction of inflammation.

2.5 Analgesic effect *in vivo*

2.5.1 Tail flick test

The Tail Flick test, originally described by Georges D'Amour and David L. Smith, was employed to evaluate spinal antinociceptive and analgesic activity [11]. Rat was placed on a Tail Flick apparatus (Panlab Harvard Apparatus LE7106 76-0293), and a focused beam of light was applied to the tail. The latency between the application of the thermal stimulus and the withdrawal of the tail was measured and recorded as the Tail Flick Latency Time (s). To prevent tissue injury and maintain tail integrity, a cut-off time of 10 seconds was set. Animals that did not respond within this period were removed from the apparatus, and a maximum latency time of 10 seconds was assigned.

2.6 Statistical analyses

Data were expressed as mean ± SEM. Statistical analyses was performed using two-way ANOVA followed by Bonferroni's multiple comparison test using GraphPad Prism software, differences were considered significant at $p < 0.05$.

3 Results

3.1 Ethnobotanical Uses

Table 1. Ethnobotanical use of *R. fruticosus.*

Part used	Preparation	Medicinal use	Mode of administration
Fruit	Decoction	Gastric pain	Oral
Leaf	Decoction	Hair tonic, Skin wounds, Respiratory infection	Oral
Brak	Decoction	Hypoglycaemia	Oral

The ethnomedicinal survey revealed that different parts of *R. fruticosus* are traditionally used for the treatment of several ailments. The fruit is mainly prepared as a decoction and administered orally to relieve gastric pain. Leaf decoctions are traditionally used as a hair tonic and for the treatment of skin wounds and respiratory infections. The bark is also prepared as a decoction and orally administered for the management of hypoglycaemia. These traditional uses highlight the therapeutic importance of *R. fruticosus* in folk medicine (Table 1).

3.2 Anti mitotic *in vitro*

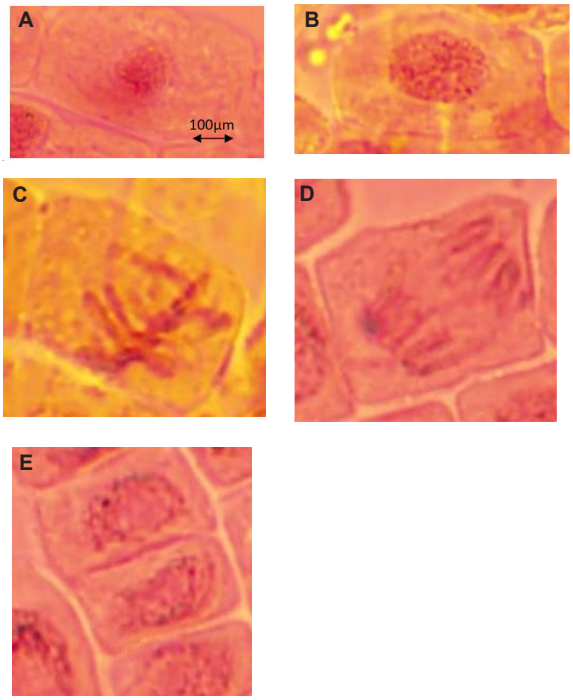

Fig. 1. Meristematic cells of *Allium cepa* illustrating the different phases of mitosis. A: Interphase; B: Prophase; C: Metaphase; D: Anaphase; E: Telophase.

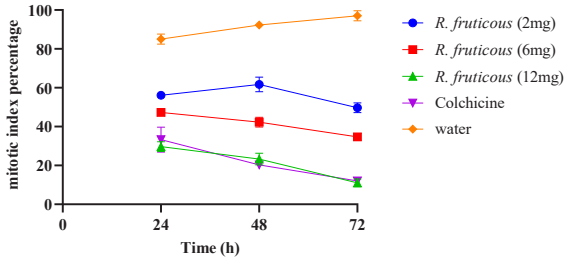

Fig. 2. mitotic index over time (24, 48 and 72 hours) for different treatments applied to onion bulb roots.

The treatments tested included increasing concentrations of *R. fruticosus* extract (2 mg/mL, 6 mg/mL, 12 mg/mL), colchicine (positive control) and distilled water (negative control).

Figure 2 shows the effect of different concentrations of *R. fruticosus* extract on the mitotic index percentage over 24, 48, and 72 h, compared with colchicine and water controls.

The water-treated group exhibited the highest mitotic index values throughout the experimental period, increasing from approximately 85% at 24 h to nearly 97% at 72 h, indicating normal cell proliferation in the absence of inhibitory treatment.

Treatment with *R. fruticosus* extract produced a concentration-dependent reduction in mitotic index percentage. The 2 mg concentration showed moderate inhibition, with mitotic index values remaining around 50–62% during the experiment. The 6 mg concentration induced a stronger inhibitory effect, progressively decreasing the mitotic index from approximately 47% at 24 h to 35% at 72 h. The 12 mg concentration exhibited the greatest antiproliferative activity among the extract-treated groups, reducing the mitotic index from about 30% at 24 h to nearly 11% at 72 h.

Colchicine, used as the positive control, also markedly reduced the mitotic index over time, confirming its well-known antimitotic activity. The inhibitory effect observed with the 12 mg extract was comparable to that of colchicine at later time points.

3.3 Anti-inflammatory *in vivo*

3.3.1 Carrageenin test

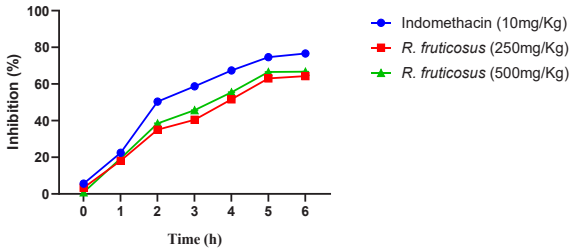

Fig. 3. Inhibitory effect of Indomethacin and aqueous extracts of *R. fruticosus* on carrageenan-induced paw edema.

The results demonstrated a progressive increase in the percentage of inhibition in all treated groups throughout the experimental period. Indomethacin (10 mg/kg), used as the reference anti-inflammatory drug, exhibited the highest inhibitory activity, increasing from approximately 5% at 0 h to nearly 77% at 6 h (see Fig. 3).

The aqueous extract of *R. fruticosus* also showed marked anti-inflammatory activity in a dose-dependent manner. The 250 mg/kg dose produced moderate inhibition, reaching approximately 64% after 6 h. In contrast, the 500 mg/kg dose exerted a stronger inhibitory effect, with inhibition values increasing progressively from 0% at baseline to about 67% at 6 h.

3.4 Analgesic effect *in vivo*

3.4.1 Tail flick test

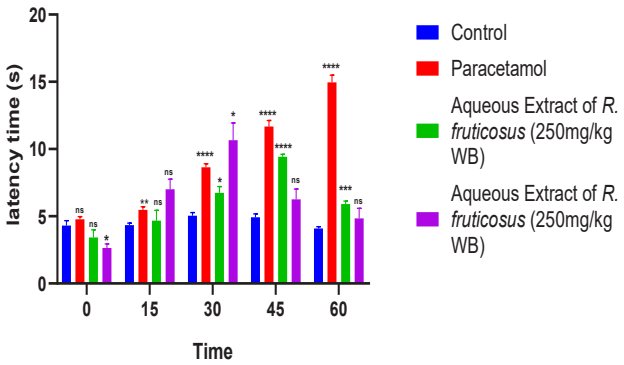

Fig. 4. Analgesic activity of the aqueous extract of *R. fruticosus*.

The analgesic activity of the aqueous extract of *R. fruticosus* was evaluated using the tail flick test and compared with the control group (water) and

paracetamol-treated group. The results demonstrated time-dependent variations in tail flick latency among the experimental groups in Fig. 4.

Baseline (0 min), no significant differences were observed between the control group and either paracetamol or the 250 mg/kg extract-treated group ($p > 0.05$). However, the second extract-treated group showed a slight but significant reduction in latency time compared with the control group ($p < 0.05$). At 15 min, paracetamol significantly increased latency time compared with the control group ($p < 0.01$), indicating an early analgesic effect, whereas both extract-treated groups did not show significant differences relative to the control group ($p > 0.05$). At 30 min, paracetamol produced a highly significant increase in latency time compared with the control group ($p < 0.0001$). The aqueous extract at 250 mg/kg also significantly increased latency time ($p < 0.05$), whereas the second extract-treated group exhibited a moderate but significant effect relative to the control group. At 45 min, the analgesic effect became more pronounced, with paracetamol and the aqueous extract (250 mg/kg) inducing highly significant increases in latency time compared with the control group ($p < 0.0001$). However, the second extract-treated group did not differ significantly from the control group ($p > 0.05$). At 60 min, paracetamol exhibited the strongest analgesic activity, showing a highly significant increase in latency time relative to the control group ($p < 0.0001$). The aqueous extract at 250 mg/kg also maintained a highly significant analgesic effect compared with the control group ($p < 0.0001$), whereas the second extract-treated group showed no significant difference. Overall, the results indicate that the aqueous extract of *R. fruticosus* possesses significant analgesic activity in the tail flick model, particularly at 250 mg/kg, with maximal effects observed between 45 and 60 min. Nevertheless, the analgesic activity remained lower than that of paracetamol, the reference analgesic drug.

4 Discussion

The present study demonstrates that *R. fruticosus* aqueous extract exhibits notable biological activity, affecting both cell division in plant systems and inflammatory and nociceptive responses in animal models. Taken together, the findings support the ethnomedicinal relevance of this species and suggest the presence of bioactive phytochemicals with antiproliferative, anti-inflammatory, and analgesic properties.

In the *Allium cepa* assay, a clear dose dependent reduction in mitotic index was observed following treatment with *R. fruticosus* extract, The mitotic index is widely recognized as a reliable indicator of cytotoxicity across living organisms [12]. The control group (distilled water) maintained a high and increasing mitotic index over time, reflecting normal and uninterrupted cell proliferation. In contrast, increasing concentrations of the extract progressively inhibited mitotic activity, with the strongest effect observed at 12 mg/mL. At this concentration, the marked decline in mitotic index over 72 hours indicates a strong antiproliferative effect, approaching the activity of colchicine, a well-established mitotic spindle inhibitor. This suggests that the extract may interfere with cell cycle progression, possibly through disruption of spindle fiber formation, DNA replication, or microtubule dynamics [13]. According to another study, the aqueous extract of *R. fruticous* reduces the mitotic index [14]. Such effects are commonly associated with phytochemicals such as tannins, and alkaloids [15], which are frequently reported in *Rubus* species [16].

In the carrageenan-induced paw edema model, the aqueous extract of *R. fruticosus* demonstrated significant anti-inflammatory activity in a dose-dependent manner. The higher dose (500 mg/kg) showed stronger activity than the lower dose (250 mg/kg), although both were less effective than indomethacin. Previous *in vivo* studies have shown that the juice of *R. fruticosus* fruits possesses anti-inflammatory activity and significantly reduces carrageenan-induced paw edema in rats, with inhibition ranging from 63% to 71% [17]. This indicates that the extract may modulate inflammatory mediators such as cytokines, which are known to be involved in carrageenan-induced inflammation. The results align with previous reports that *R. fruticosus* contain Anthocyanin with cyclooxygenase (COX) inhibitory and antioxidant properties, both of which contribute to reduced edema formation [18].

The analgesic assessment using the tail flick test further confirmed the pharmacological potential of the aqueous extract of *R. fruticosus*. The 250 mg/kg dose produced a significant increase in latency time, particularly between 30 and 60 minutes. The delayed but sustained response compared to paracetamol suggests a slower onset of action but a measurable analgesic effect [19]. The reduced or inconsistent response at the higher dose may indicate a biphasic effect or possible receptor saturation, which is not uncommon in crude plant extracts due to the presence of multiple interacting constituents.

Overall, the combined anti-inflammatory and analgesic effects suggest that *R. fruticosus* may exert its action through overlapping mechanisms, possibly involving inhibition of prostaglandin synthesis and modulation of oxidative stress pathways [20]. These findings provide pharmacological support for its traditional use in treating pain, and inflammatory conditions.

However, while the results are promising, they are limited to crude extract testing and preclinical models. The specific bioactive compounds responsible for the observed effects were not isolated, and the exact molecular mechanisms remain unclear. Further studies involving phytochemical characterization, fractionation, and molecular assays are necessary to identify active constituents and confirm their mechanisms of action. Toxicological evaluations are also essential to determine safe dosage ranges, especially given the observed strong inhibition of cell division at higher concentrations.

5 Conclusion

R. fruticosus aqueous extract exhibits significant antiproliferative, anti-inflammatory, and analgesic activities in experimental models, supporting its traditional medicinal use. These findings justify further investigation into its therapeutic potential and possible development of novel plant-derived pharmacological agents.

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