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Comprehensive Profiling of Phytochemical Estimation and Anti Nociceptive Effect of Malus Pumila

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ABSTRACT

Malus pumila, commonly known as apple, is a widely consumed fruit with a long history of medicinal use. This study aimed to provide a comprehensive profile of the phytochemical constituents present in Malus pumila and evaluate its anti-nociceptive potential. Various phytochemical analyses were conducted to identify the presence of bioactive compounds, including polyphenols, flavonoids, terpenoids, and alkaloids, in different parts of Malus pumila, such as the fruit peel, flesh, and seeds. Furthermore, the anti-nociceptive effect of Malus pumila extracts was assessed using in vivo models of pain. The results showed a significant reduction in nociceptive responses in mice treated with Malus pumila extracts, indicating its potential analgesic properties. These effects were dose-dependent, suggesting that Malus pumila may serve as a natural remedy for pain management. The findings of this study contribute to a better understanding of the phytochemical composition of Malus pumila and its potential as an anti-nociceptive agent. Further research is warranted to elucidate the mechanisms of action and safety profile of Malus pumila extracts for future development as a therapeutic option for pain management.

Keywords: Malus Pumila, Phytochemical Composition, Anti-Nociceptive Agent, Vivo Models.

INTRODUCTION

People who have lost their pain function appear to experience recurring injuries such as burns, fractures, and self-injuries. The International Association for the Study of Pain (IASP) defines pain as an unpleasant sensory and emotional experience that is related with or explained in terms of actual or potential tissue damage. In humans, pain has three dimensions: sensory discrimination, motivational-affective, and cognitive-evaluative [1]. Study of plant species that are used in traditional herbal medicine as pain killers therefore form a logical search strategy for new analgesic drugs [2]. For a number of reasons, compounds generated from medicinal extracts are attractive: they are frequently multi- or macrocyclic, stereo chemically complicated molecules with little chance of prior chemical synthesis, and they frequently exhibit intriguing biological features. Perhaps most crucially, though, is that parent extracts have been "clinically" examined in their customary settings for millennia in certain situations [3]. Anti-nociceptive medications are used to manage pain. Since ancient times, the Unani medical system has used a medicinal plant that is the primary source of herbal pharmaceuticals used by the majority of the world's population. It encompasses the 70–80% of the global population that uses herbal medicine. This information has been passed down from one generation to the next. The plants that have been shown to be pharmacologically and therapeutically effective now need to be documented. There are several efficient anti-nociceptive medications on the market, however they might have negative side effects like heartburn and stomach ulcers [4]. Nociceptor control primary sensory neurons' excitability and sensitization through signal-transduction pathways. The sensory nerve ending is stimulated by noxious stimuli through physical, chemical, or thermal means. This results in the opening of ion channels, which permits the entry of cations and depolarization. While K+ and Ca2+ help regulate the excitability of neurons, Na+ channels are required for the production and conduction of action potentials. Peripheral information processing is facilitated by several significant receptors, including acid-sensing ion channels and transient receptor potential (TRP) receptors. Signal transduction is also significantly influenced by inflammatory mediators including prostaglandin and bradykinin. A delicious, edible fruit that apple trees bear is called an apple. Everywhere in the world, apple trees are grown.

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Asia and Europe were the tree's original home. Apples have been traditionally used to cure a variety of conditions, such as warts, diabetes, scurvy, fever, diarrhoea, and constipation. Apple consumption, however, appears to be protective against cancer, especially colorectal and lung cancer, though there may be other types as well. It may also have positive effects on pulmonary function, which may prevent asthma, and cardiovascular disease by reducing cardiovascular risk factors. The antioxidant and anti-inflammatory properties of apples may also be beneficial for a number of other illnesses, according to early research [5].

MATERIAL AND METHOD

Collection of Plant Materials and Authentication of Plant Part

Malus pumila leaves were gathered in the Himalayan region of Uttarakhand, India. The plant's herbarium was kindly made, and it was sent for identification to the Botany Department of Safia College of Science, Bhopal, India. Dr. Zia-Ul-Hasan, Head of the Botany Department at Safia College of Science, Bhopal, India, verified the authenticity of the plants.

Plant authentication number obtained was **112/Bot/Saf/18.**

Extraction of malus pumila leaves

Dried pulverised leaves of Malus pumila were sulphurized using Petroleum ether as a non-polar solvent. Exhausted plant material was dried and extracted with methanol, with colourless solvent collected for confirmation and residual solvent evaporation [6].

PHYTOCHEMICAL INVESTIGATION

Phytochemical screening was carried out using established protocols to identify various Phyto-constituents, namely alkaloids, terpenoids, glycosides, steroids, triterpenoids, flavonoids, carbohydrates, phenolic substance, saponins, and tannins in different extracts [7][8].

TOXICITY STUDY

Acute oral toxicity study (OECD 423)

The study followed OECD guidelines for acute oral toxicity in nulliparous healthy female mice. After administering an extract, the mice were observed for 14 days, observing

behaviour changes, tremors, convulsions, and more. Clinical signs were noted, and all animals were euthanized. [9]

Approval of animals

Institutional Animal Ethics Committee (IAEC) of PBRI, Bhopal has approved all animal experiments with CPCSEA **(Reg. No.1824/PO/ERe/S/15/CPCSEA).**

Protocol approval reference number is **PBRI/IAEC/PN-18013.**

RESULTS AND DISCUSSION

Plant Extraction

The plant material was extracted using a soxhlet equipment, and the yield percentage determined by the formula was found to be 9.43% for methanol and 0.41 % for petroleum ether.

Acute Oral Toxicity

The OECD 423 standards were followed in the conduct of the acute oral toxicity investigation. For the toxicity experiments, four dose ranges—5 mg/kg, 50 mg/kg, 300 mg/kg, and 2000 mg/kg—were used, and no death was noted.

Table 1 –Acute oral toxicity

Groups	Observations/ Mortality
5 mg/kg Bodyweight	0/3
50 mg/kg Bodyweight	0/3
300 mg/kg Bodyweight	0/3
2000 mg/kg Bodyweight	0/3

PHYTOCHEMICAL ANALYSIS OF *MALUS PUMILA* LEAVES EXTRACT

Table 2- Phytochemical analysis of *Malus pumila* methanolic leaves extract

S. No.	Test	Result	S. No.	Test	Result
1	**Alkaloids**	**Absent**		Shinoda test	Pass

	Mayer's reagent test	Fail		Lead acetate test	Pass
	Wagner's reagent test	Fail	**5**	**Glycoside**	**Present**
	Hager's reagent test	Fail		Borntrager	Pass
2	**Carbohydrate**	**Present**		Legal's test	Pass
	Molish's test	Pass		Killer-killiani test	Pass
	Fehling's test	Pass	**6**	**Tannin and phenolic compound**	**Present**
	Benedict's test	Pass		Ferric chloride test	Pass
	Barfoed' test	Pass		Lead acetate test	Pass
3	**Protein and amino acids**	**present**		Dilute iodine solution	Pass
	Biuret test	Pass	**7**	**Saponin**	**Absent**
	Million's test	Pass		Foam test	Fail
	Ninhydrin's test	Pass	**8**	**Test for triterpenoids and steroids**	**Present**
4	**Flavanoids**	**Present**		Salwonki test	Pass
	Alkaline reagent test	Pass		Libberman and burchard's test	Pass

IN VITRO ANTIOXIDANT ANALYSIS

Hydrogen peroxide Scavenging Assay

Table 3-Hydrogen peroxide assay of Ascorbic acid-

Concentration in µg/ml	% inhibition	IC 50 Value
20 µg/ml	51.47808	**17.95**
40 µg/ml	56.88073	
60 µg/ml	62.69113	
80 µg/ml	70.33639	
100 µg/ml	77.47197	

FFig.1-Hydrogen peroxide scavenging activity of the methanolic extracts of m.pumila

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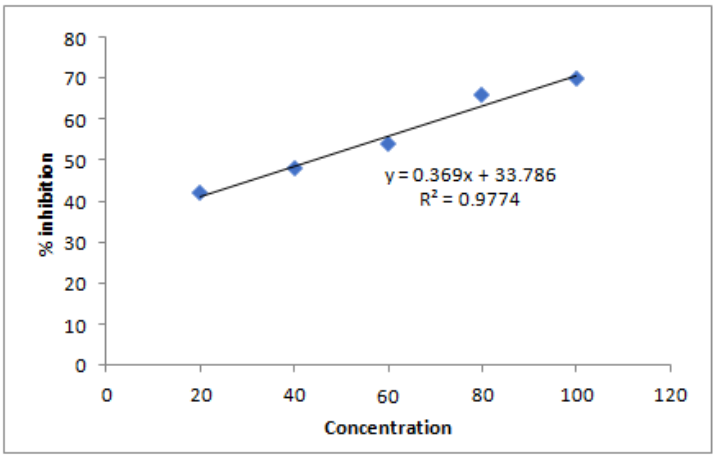

Table 7.4- Hydrogen peroxide assay of methanolic leaves extract of *Malus pumila*

Concentration in µg/ml	% inhibition	IC 50 Value
20 µg/ml	44.34251	**38.06**
40 µg/ml	49.43935	
60 µg/ml	58.10398	
80 µg/ml	64.8318	
100 µg/ml	69.72477	

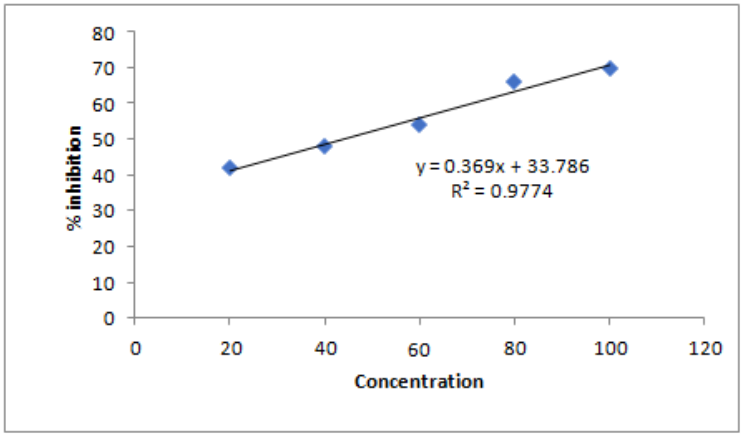

Fig. 7.2-hydrogen peroxide Assy of Malus pumila

Ascorbic acid (standard) at different concentrations (20, 40, 60, 80, and 100 ug/ml) in the hydrogen peroxide scavenging assay shows the percent inhibition as 44.34, 49.43, 58.10, and

69.72, respectively. The IC50 values of ascorbic acid and the methanolic leaves extract of Malus pumila are 17.95 and 38.06, respectively.

IN VIVO ANTI NOCICEPTIVE ACTIVITY

Table 7.5- Formalin induced lick test

Treatment group	Mean lick time (sec) ± SD	
	Early Phase	Late phase
Control	102 ± 4.358	240.5±26.113
Standard	28.5 ± 3.593	37.16±2.339
MPME200	82.5 ± 4.958	132 ±3.696
MPME400	35.66 ± 2.94	45.33±3.543

There are two stages of formalin-induced behaviour that are related to both acute and tonic pain. The ability of morphine to influence the late phase was studied, and it was given systemically either before or after the early phase. When morphine was delivered right after the early phase, its inhibitory effects were noticeably more potent than when it was administered beforehand. It seems that morphine's effects on the late phase are limited by certain neuronal and/or behavioural alterations that occur during the early phase. The formalin-induced licking number was dose-dependently inhibited by MPME in both formalin test phases. At all experimental dosages, the effect is substantial ($p < 0.001$); the dose of 400 mg/kg of MPME resulted in 35.66 ± 2.94 licking in the first phase and 45.33 ± 3.54 in the second. Similarly, at all experimental dosages, the effect is significant; at 200 mg/kg of MPME, licking was detected in the first phase at 82.5 ± 4.958 and in the second phase at 132 ± 3.696. As a result, oral treatment of the usual medication along with both extracts at 200 and 400 mg/kg significantly reduced the amount of formalin-induced licking in a dose-dependent manner as compared to the control.

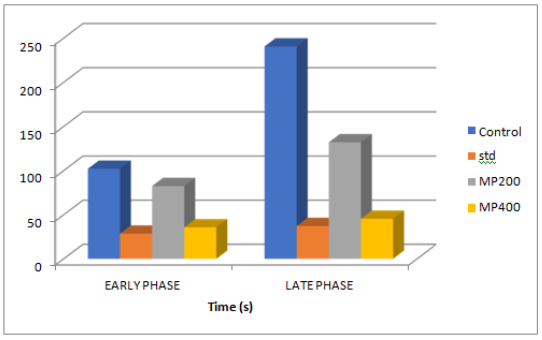

Fig. 7.3-Formalin induced lick test

TAIL IMMERSION TEST

Table 7.6: Pre-treatment observations of tail immersion test

Treatment	Observations
Control	1.33± 0.745
Standard	3.16±0.687
MPME200	0.816±0.816
MPME400	1.83±0.372

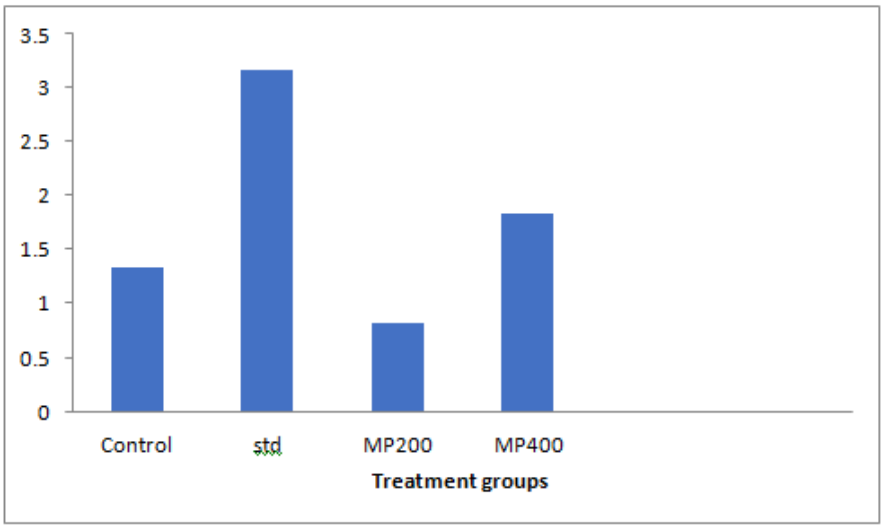

Fig. 7.4 - Pre-treatment observations

Table 7.7: Post treatment with leaves extract of *Malus pumila*at reactiontime 60, 90, 120 and 150 min.

Treatment	60 min	90 min	120 min	150 min
Control	1.5± 0.5	1.66±0.74	1.25±0.901	1.8±0.786
Standard	36.16± 4.41	40.16±4.59	41.33± 3.636	37.83± 3.236
MPME200	8.5 ± 0.763	15.16± 2.266	20 ± 2.081	15± 1.414

MPME400	16.33± 1.247	20 ± 1.29	35.83± 2.409	22.83± 1.343

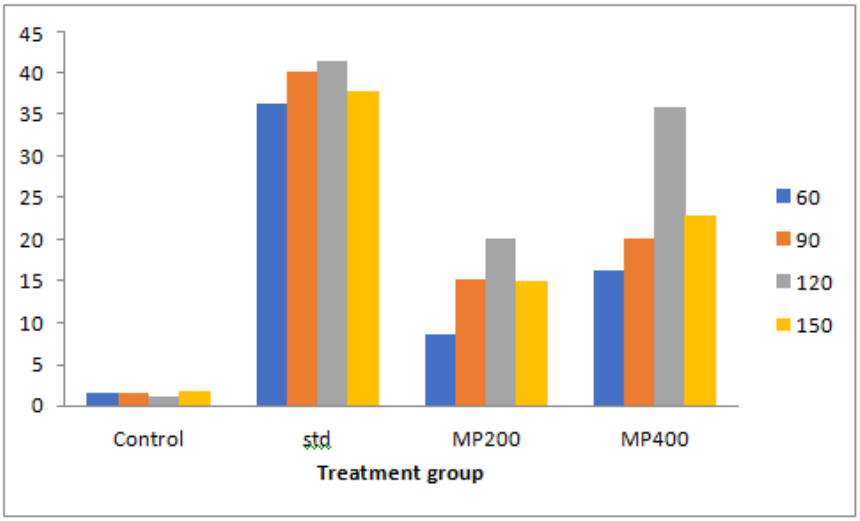

Fig. 7.5 - Post treatment with leaves extract of *Malus pumila*at reaction time

MPME demonstrated significant, dose-dependent antinociceptive activity in the tail immersion test. Specifically, the 200 mg/kg and 400 mg/kg oral MPME dosages both markedly delayed the reaction time to a nociceptive stimuli at 60 minutes following delivery. Both the MPME extract and the reference medication, morphine, demonstrated potent antinociceptive effects in a dose-dependent manner.

CONCLUSION

The study evaluates the antinociceptive activity of the methanolic extract of leaves of Malus pumila in various pain models. The results suggest that the extract may be due to blocking inflammatory mediators or eicosanoid system receptors. The tail immersion method and the tail flick model were used to test the central antinociceptive activity. The results show that the Malus pumila Methanolic Extract has antinociceptive properties by acting on both supra spinal and spinal receptors. Further research is needed to determine the exact chemical substance present in the extract. The results support the traditional use of MPME in some painful conditions, mediated partly by opioid receptors.

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