Analgesic Activity of Ethylacetate Fraction from Methanolic Extract of *Daucus Carota* Seeds in Rats

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ABSTRACT

Medicinal plants are a rich source of bioactive compounds with analgesic and anti-inflammatory potential. *Daucus carota* (carrot) seeds contain phenolics and flavonoids, which are known to contribute to antioxidant and pain-modulating effects. The present study aimed to investigate the phytochemical composition and analgesic activity of the ethylacetate fraction of the methanolic extract of *Daucus carota* seeds. Seeds were defatted with petroleum ether and extracted with methanol using Soxhlet apparatus. The ethylacetate fraction was subjected to phytochemical screening, TLC analysis, and quantification of phenolics and flavonoids using Folin-Ciocalteu and aluminium chloride methods, respectively. Analgesic activity was evaluated in Wistar rats using tail flick and hot plate methods at doses of 50 and 100 mg/kg, with ibuprofen as the standard drug. Phytochemical analysis confirmed the presence of phenolics, tannins, flavonoids, saponins, and carbohydrates. The ethylacetate fraction showed high phenolic (98.08 ± 0.916 mg GAE/g) and flavonoid (124.17 ± 3.441 mg QE/g) content. In both tail flick and hot plate models, the fraction significantly increased reaction times compared to control, demonstrating analgesic activity comparable to ibuprofen. The ethylacetate fraction of *Daucus carota* methanolic extract exhibits significant analgesic activity, likely mediated via central mechanisms. These findings support the traditional use of carrot seeds in pain management and warrant further studies for isolation and characterization of active constituents.

Keywords: *Daucus carota*, Analgesic, Tail flick, Hot plate, fractionation

INTRODUCTION

Medicinal plants have long been recognized as a valuable source of bioactive compounds with therapeutic potential, particularly in the management of pain and inflammation. Natural products such as alkaloids, flavonoids, tannins, and phenolics are widely reported to possess significant analgesic and anti-inflammatory properties. Phytochemical screening serves as a fundamental step in identifying these compounds and correlating them with pharmacological activity. Extraction and fractionation techniques, including Soxhlet extraction and column chromatography, are commonly employed to isolate bioactive constituents.

Among medicinal plants, *Daucus carota* (carrot) seeds have attracted attention due to their rich phytochemical profile, including phenolics and flavonoids, which are known to contribute to antioxidant and analgesic effects[1]. The evaluation of analgesic activity using experimental models such as the tail flick and hot plate methods provides insight into the central and peripheral mechanisms of pain modulation[2]. These models are well established for screening centrally acting analgesics, as they assess both spinal reflexes and supraspinal responses to nociceptive stimuli[3,4]. Ibuprofen, a standard non-steroidal anti-inflammatory drug (NSAID), is often used as a reference in such studies to validate the efficacy of plant extracts[5].

The present study was undertaken to investigate the phytochemical composition and analgesic potential of the ethylacetate fraction of the methanolic extract of *Daucus carota* seeds. By quantifying phenolic and flavonoid content and evaluating analgesic activity in Wistar rats, the study aims to establish a scientific basis for the traditional use of carrot seeds in pain management.

MATERIAL AND METHODS

The seeds of *Daucus carota* was purchased from Indianjadibooti e-commerce platform. The taxonomical identification and authentication of the plant material was done at RB Science, Bhopal. Petroleum ether, Chloroform, Methanol, Ethyl acetate, Glacial acetic acid, Acetone, Formic acid, Benzene, N-propanol, Ethanol, Tween-80, Dimethylsulphoxide (DMSO), Glycerine, conc. Sulphuric acid, Hydrochloric acid, Benzene, N-Butanol, Dichloromethane, n-hexane, pyridine, toluene, xylene, aniisaldehyde, *α*-napthol, bismuth carbonate, calcium chloride, copper sulphate, Ferric chloride, Follin's reagent, Iodine, Lead acetate, Magnesium chloride, Mercuric chloride, Ninhydrin, Nitric acid, Phloroglucinol, Potassium iodide, Potassium Dichromate, Potassium sodium Tartarate, Ruthenium red, Safranine, Sodium acetate, Sodium iodide, Sodium hydroxide, Sodium nitroprusside. All the chemicals (S.D Fine Chemicals Pvt. Ltd. Mumbai) were purchased from local supplier and were of analytical grade.

Extraction and phytochemical screening

The procured seeds of *Daucus carota* was dried in shade and then powdered with a mechanical grinder. The powdered plant material was subjected to extraction with petroleum ether (60-80°C) in a soxhlet apparatus. The extraction was continued till the defatting of the material had taken place. The defatted plant powder was weighed (86 g) and was evenly packed in a paper thimble and placed in extractor of the soxhlet apparatus and extracted with methanol (400 mL) by hot continuous extraction process for about 10 h. The extract was filtered hot through muslin cloth to remove impurities and concentrated using rotary vacuum evaporator to $1/10^{th}$ of its original volume. The concentrated extract was transferred to a beaker and were evaporated on the water bath. The dried extract was placed in desiccator for complete drying and the dried extract was packed in air tight container for the further studies.[6]

The preliminary phytochemical studies were performed for testing different chemical groups present in the solvent extract. General screening of the extracts of the plant materials carried out for qualitative determination of the groups of organic compound present in them.[7]

Fractionation of ethanolic extract

Slurry of activated silica gel was prepared in ethanol and was poured into the column with the help of a hollow glass cylinder. While pouring the slurry the column was continuously tapped with a rubber cork so that a compact column was formed devoid of any air bubble. The solvent was eluted thereafter at a steady rate till solvent head remained about 2-3 cm above the column. About 1 g of extract was dissolved in 1mL of methanol and placed on the top of the silica bed in the column using a Pasteur pipette. Thereafter appropriate solvent (ethylacetate) was poured into the column for elucidation of components. Elute was collected at the rate of 20 drops per minute & solvent fraction was of about 50 ml. The fraction was subjected to TLC on silica gel G. The fraction was analyzed by TLC using chloroform-methanol (9:1) as the solvent for testing of flavonoids.

Quantification of phenolics in the ethylacetate fraction

For total phenolic content determination, 200 µL of sample was mixed with 1.4 mL purified water and 100 µL of Folin-Ciocalteu reagent. After at least 30 s (but not exceeding 8 min), 300 µL of 20% Na_2CO_3 aqueous solution was added and the mixture allowed to stand for 2 h. The absorbance was measured at 765 nm with a UV-Vis spectrophotometer. Standard solutions of gallic acid (10-100 ppm) were similarly treated to plot the analytical curve. The control solution contained 200 µL of methanol and suitable reagents, and it was prepared and incubated under the same conditions as the rest of the samples. Results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of the dry sample.[8]

Quantification of flavonoids in the ethylacetate fraction

Determination of total flavonoids content was based on aluminium chloride method. 50 mg quercetin was dissolved in 50 ml methanol, and various aliquots of 25- 150µg/ml were prepared in methanol. 0.1 g of

ethylacetate fraction was extracted with 10 ml methanol, filtered, and make up the volume up to 100 ml. One ml (1mg/ml) of this extract was for the estimation of flavonoid. 1 mL of 10% sodium nitrite solution was added to 1 mL of extract or quercetin solution and allowed to stand for 10 min. To this was added 1 ml of 2% $AlCl_3$ methanolic solution and allowed to stand for 60 min at room temperature; absorbance was measured at 420 nm.[9]

Pharmacological Study

Healthy Wistar rats of either sex, weighing 180-250g were used for the study. The animals were housed in cages during the course of experimental period and maintained at 12 day and night schedule with a temperature [17-26°C] maintained at standard experimental condition. The animals were fed with standard rodent pellet feed and water *ad libitum*. The animals were fasted 12 hours before the experiment with free access to only water.

Tail flick method

The analgesic activity was evaluated using tail flick method.[10] Animals were divided into 4 groups (n = 6) as follows: Group -- I - Control - treated with vehicle (normal saline); Group -- II - Standard drug – Ibuprofen; Group – III– *Daucus carota* ethylacetate fraction (DCEAF) was administered in dose of 50 mg/kg; Group – IV– *Daucus carota* ethylacetate fraction (DCEAF) was administered in dose of 50 mg/kg

About 5 cm from the distal end, tail of each rat was immersed in warm water maintained at 50°C. The reaction time (in seconds) was the time taken by the rat to flick its tail due to pain. The first reading was omitted and reaction time was taken as the average of the next two readings. The reaction time was recorded before (0 min) and at 15, 30, 45, and 60 min after the administration of the treatments. The maximum reaction time was fixed at 15 sec to prevent any tail tissue injury. If the reading exceeds 15 sec, it would be considered as maximum analgesia. The maximum possible analgesia (MPA) was calculated as follows:

$$MPA = \frac{\text{Reaction time for treatment} - \text{reaction time for saline}}{15\ \text{sec} - \text{reaction time for saline}}\ X\ 100$$

Hot Plate method

Animals were divided into 4 groups (n = 6) as follows: Group -- I - Control - treated with vehicle (normal saline); Group -- II - Standard drug – Ibuprofen; Group – III– DCEAF was administered in dose of 50 mg/kg; Group – IV- DCEAF was administered in dose of 100 mg/kg

The rats were placed on a hot plate maintained at 55°C within the restrainer. The reaction time (in seconds) or latency period was determined as the time taken for the rats to react to the thermal pain by licking their paws or jumping. The reaction time was recorded before (0 min) and at 15, 30, 45, and 60 min after the administration of the treatments. The maximum reaction time was fixed at 45 sec to prevent any injury to the tissues of the paws. If the reading exceeds 45 sec, it would be considered as maximum analgesia. The maximum possible analgesia (MPA) was calculated as follows:[11]

MPA = Reaction time for treatment − reaction time for vehicle/45 sec- reaction time for vehicle x 100

RESULTS AND DISCUSSION

The yield of the plant extract obtained from methanol solvent was 11.40% and the results obtained from phytochemical testing of the methanolic extract of the plant of *Daucus carota* revealed the presence of phenolics, tannins, flavonoids, saponin and carbohydrates in the extract (Table 1).

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Table 1. Phytochemical analysis of methanolic extract

Chemical Tests	Inference
Mayer's reagent	-
Hager's reagent	-
Wagner's reagent	-
Dragendorff's reagent	-
Baljet test	+
Legal's test	+
Keller-Kiliani	-
Ferric chloride	+
Gelatin Solution	+
Lead acetate test	+
$FeCl_3$ test	+
Alkaline reagent test	+
Shinoda test	+
Foam test	-
Hemolytic test	-
Lead acetate	-
Spot	-
Saponification	-
Water	-
Molish test	+
Fehling's solution test	+
Benedict's test	+

(+) Indicates 'Presence'; (-) Indicates 'Absence'

The ethylacetate fraction from the methanolic extract was found to be rich in phenolics, tannins and flavonoids.

The total phenolic content of ethylacetate fraction of the methanolic extract of *Daucus carota* was calculated to be 98.08 ± 0.916 GAE mg/g. The total flavonoid content of ethylacetate fraction of the methanolic extract of *Daucus carota* was calculated to be 124.17 ± 3.441 QE mg/g.

Pharmacological Action of solvent fractions

The ethylacetate fraction was assessed for analgesic effect in rats using hot plate and tail flick methods at two doses (50 and 100 mg/kg). Analgesics are drugs that act on peripheral or central nervous system to selectively relieve pain without significantly altering consciousness.[12] Centrally acting analgesics act by raising the threshold for pain and also altering the physiological response to pain. On the other hand, peripherally acting analgesics act by inhibiting the generation of impulses at chemoreceptor site of pain.[13] The animal models employed for screening of analgesic activity in this study are pain-state models using thermal stimuli which

include tail-flick and hot plate methods. Both methods are useful in illustrating centrally mediated antinociceptive responses which focus generally on changes above the spinal cord level.[14] While the tail-flick method mediates a spinal reflex to a nociceptive stimulus, hot plate method involves higher brain functions and is regarded a supraspinally organized response.[15] The animal model used for screening of analgesic activity in this study is pain-state model using thermal stimuli which includes tail-flick method. The tail-flick method is known to mediate a spinal reflex to a nociceptive stimulus.[16] Ibuprofen was used as the reference drug, which is considered as mild analgesic.

Tail flick method

The results of analgesic activity of ethylacetate fraction of *Daucus carota* methanolic extract by tail flick method are shown in table 2. Rats treated with normal saline (vehicle control) did not exhibit any significant difference in the response time on tail-flick throughout the 60 min duration of observation. The duration of response time in Ibuprofen and solvent fractions was significantly higher as compared to the saline treated animals. The highest reaction time for TPHF and TPEAF was 6.51 ± 0.242 sec and 5.52 ± 0.66 respectively at 30 min post administration while it was 3.43 ± 0.095 sec and 6.28 ± 0.171 sec for vehicle treated group and Ibuprofen respectively at the same time duration (Figure 1).

Table 2. Effect of solvent fractions on tail flick response

Group	Response Time in seconds				
	0 min	15 min	30 min	45 min	60 min
Vehicle Control	3.14 ± 0.055	3.24 ± 0.055	3.44 ± 0.055	3.40 ± 0.071	3.48 ± 0.084
Ibuprofen	4.22 ± 0.130	6.10 ± 0.453	6.22 ± 0.164	6.80 ± 0.141	7.96 ± 0.336
DCEAF 50	3.50 ± 0.143	4.38 ± 0.349	6.16 ± 0.230	6.02 ± 0.110	5.88 ± 0.084
DCEAF 100	3.90 ± 0.173	4.64 ± 0.344	6.38 ± 0.192	6.12 ± 0.130	6.04 ± 0.089

Values are mean ± SD (n = 6)

The tail-flick method is based on the observation that morphine-like compounds are selectively able to prolong the response time of typical tail-withdrawal in rats in response to pain stimulus. Analgesic drugs which are centrally acting elevate pain threshold of animals towards heat and pressure.[17]

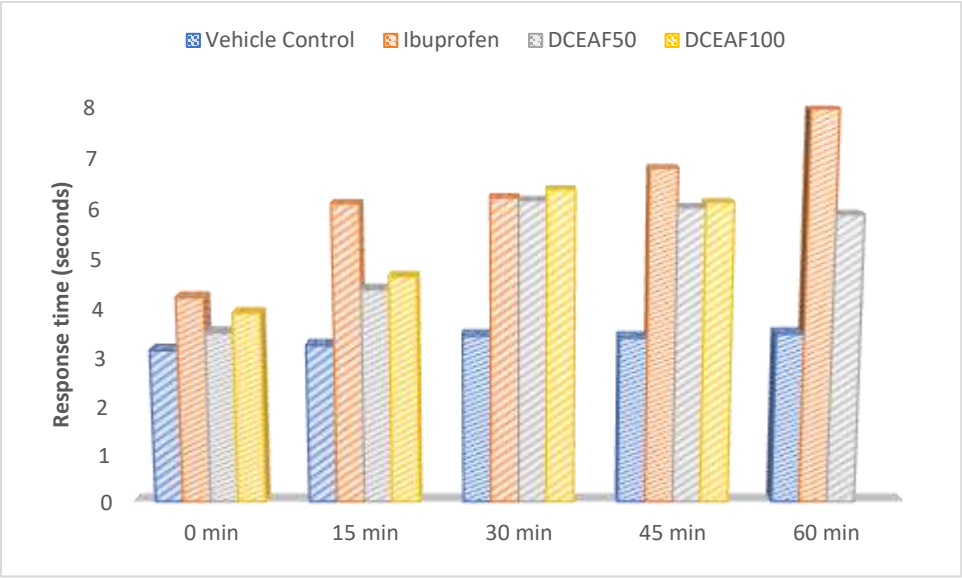


Figure 1. Graphical comparison of analgesic activity of ethylacetate fraction

Hot plate method

The results of analgesic activity of ethylacetate fraction of *Daucus carota* methanolic extract by hot plate method are shown in table 3. Rats treated with normal saline (vehicle control) did not exhibit any significant difference in the response time during the 60 min duration of observation.

Table 3. Effect of solvent fractions on hot plate

Group	Reaction Time in seconds				
	0 min	**15 min**	**30 min**	**45 min**	**60 min**
Vehicle Control	28.17 ± 3.312	24.67 ± 2.658	29.50 ± 2.168	24.17 ± 2.041	26.83 ± 1.330
Ibuprofen	32.67 ± 3.077	38.50 ± 1.975	40.17 ± 3.545	43.50 ± 2.881	43.67 ± 2.160
DCEAF 50	30.83 ± 2.137	32.00 ± 2.829	35.67 ± 2.582	38.67 ± 1.751	38.83 ± 2.563
DCEAF 100	31.50 ± 2.881	31.83 ± 2.483	35.83 ± 2.483	39.17 ± 2.787	40.17 ± 1.941

Values are mean ± SD (n = 6)

The difference in the mean reaction time of the extract and the control groups was statistically significant during all observation times. Analgesia in ibuprofen as well as the ethylacetate fraction from the methanolic extract of *Daucus carota* treated rats was only detectable at 30, 45 and 60 min (Figure 2). Hot plate method produces two measureable behavioural components in response to thermal pain, with regard to their reaction times. Responses such as paw licking and jumping in rats are considered to be supra-spinally integrated.

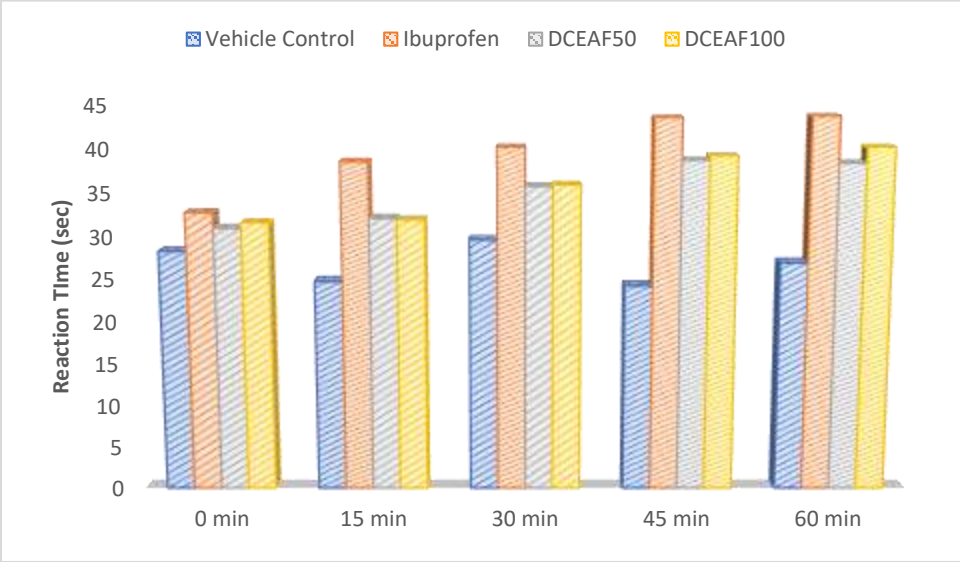


Figure 2. Comparison of reaction time in hot plate method

A number of alkaloids, flavanoids, steroids, and tannin isolated from medicinal plants have been reported to possess significant analgesic activity. The presence of flavonoids, phenolics and tannins in the solvent fractions would be responsible for their analgesic responses.

CONCLUSION

It can be concluded form the study that the ethylacetate fraction from the methanolic extract of plant *Daucus carota* has significant analgesic effect in tail flick and hot plate method equivalent to the standard drug (ibuprofen). It can be assumed that the extract might be acting via the central nervous system. Further work

related to the characterization of the active constituents as well as evaluation of the mechanism of antitussive effect will be carried out in our laboratory in the near future.

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