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Original Research Article

Assessment of Analgesic, Antioxidant, and Antiinflammatory potential of *Citrus maxima* (Burm.) Merr. Seed solvent fractions on *Swiss albino* Animal Model

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ABSTRACT

Citrus maxima has been utilized in folk medicine for its various pharmacological characteristics. *In-vivo*and*in-vitro*researchhave been conducted on the methanolic extract of seed. Thus, there is a possibility that some bioactive substances maybe present in the methanolic solvent fractions. N-hexane and dichloromethane were used to fractionate the crude methanolic extract. The objective was to analyze the analgesic, anti-inflammatory, and antioxidant potentials of the dichloromethane (DMS) and n-hexane (NHS) fractions. The writhing test by inducing acetic acid and paw-licking method by inducing formalin were utilized to evaluate the analgesic potential. The 2, 2-diphenyl-1-picryl hydrazyl (DPPH) and hydrogen peroxide scavenging assay were used to measure the antioxidant effect. The paw edema test inducing formalin and carrageenan utilized for assessing the anti-inflammatory efficacy. NHS showed a significance in decreasing the writhing and considered to have significant analgesic effect. Compared to the higher (400 mg/kg) dose (**P<0.01), the lower (200 mg/kg) dose had a smaller effect (***P<0.001). At lower doses, DMS demonstrated a minor effect (**P<0.01) and at higher doses, a moderate effect on writhing reduction. NHS demonstrated a lower concentration-dependent scavenging potential of free radicals. At higher concentrations, DMS had a strong antioxidant activity with IC_{50}valuesignificantly lower than that of NHS. In a dose-dependent way, NHS exhibited a considerable high potential (**P<0.01) in reducing paw edema. The current study may provideevidence that the methanolic fractions of seeds could serve as a viable replacement for naturally occurring analgesic, antioxidant, and anti-inflammatory substances.

Keywords: *Citrus maxima*, Analgesic, Antioxidant, Antiinflammatory, Seed, Fraction, *Swiss albino*, n-hexane, Dichloromethane.

Introduction

The utilization of alternative therapy has been historically prevalent in underdeveloped nations such as Bangladesh, China, and India. Approximately 25% of medications given in underdeveloped nations are composed of plant-derived components.[1]Since the majority of the population of world dwells in underdeveloped nations, a sizeable segment of that population relies on medications derived from natural sources for their primary healthcare requirements.[1] Plants possess a multitude of chemical components that form the basis for medicinal therapies globally, due to their therapeutic capabilities and minimal side effects.[2,3]Numerous underdeveloped countries rely on traditional medication for its accessibility and cost-effectiveness, but researchers globally consider medicinal plants as prospective sources of novel chemical compounds.[4]*Citrus maxima* originate from Asia and is cultivated for commercial purposes in countries such as China, Nepal, India, Bangladesh,etc.[5,6]The plant flourishes in areas with temperatures ranging from 25-32°C and rainfall 1500-2500mm in a three to four months dry season. The plant grows well in coarse sand to dense clay but prefers nutrient-rich or fertile soil. [7]The plant contains numerous organic compounds. Neronyl acetate, limonin, geraniol, and nerolol can be found in the essential oils extracted from leaves and immature fruits. [8]Research on *Citrus maxima* extracts has demonstrated their efficacy in addressing microbiological diseases, oxidative stress, diabetes, liver damage, inflammation, algesia, and as a treatment for central nervous system depressants, among other conditions, in animal studies.[9]The pomelo peel constitutes around thirty percent of the actual weight of the fruit and consist of secondary metabolites including aroma-active volatile constituents, phenolic acids, pectin, flavonoids, carotenoids, coumarins, and polysaccharides.[10]The efficacy of fruit juice is used in weight loss and lowering cholesterol levels. The essential oil derived from *Citrus maxima* is utilized in personal care products. The leaves are utilized for epilepsy, chorea, and severe chronic cough, while the flower serves as a sedative for anxiety.[11]The methanol extract of the seed has anxiolytic, analgesic, antioxidant, antidiabetic, anthelmintic, andanti-inflammatory properties. [8,12]The present study aims at assessing the analgesic, antioxidant, and anti-inflammatory effects of the partitioned solvent fractions derived from the *Citrus maxima* seeds. This work may potentially provide a basis for future preliminary chromatographic analyses aimed at isolating various polar and non-polar chemicals from this seed component. The results may also aid future molecular docking investigations, improving our comprehension of the pharmacological potential of *Citrus maxima* fractions.

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Materials and Methods

Chemicals

Diclofenac sodium and Diazepam were acquired from Square Pharmaceuticals Ltd located in Gazipur. From Eskayef Pharmaceuticals Ltd. Indomethacin was procuredthat is Located in Tongi, Bangladesh. Morphine sulfate was acquired from Aristopharma Ltd. in Dhaka, Bangladesh. N-hexane and Dichloromethane were bought from Sigma-Aldrich based in Hamburg, Germany. Acetic acid, Carrageenan and Formalin were procured from local vendors. Analytical-grade compounds and reagents were employed in this investigation.

Processing and Collection of plant material for extraction

The seeds of *Citrus maxima* were acquired from an indigenous plant vendor at the Bangladesh Forest Research Institute (BFRI) in Chattogramon the year 2019, April. Taxonomist Dr. Sheikh Bokhtear Uddin, from the Department of Botany at the University of Chittagong, verified the seeds and provided the voucher specimen number Accn No. SBU of extraction of plant sample 5380. After washing and shade-drying the seeds, they were converted into a powder form using a grinding machine. 1170 g of powdered seed was macerated in 3 liter of methanol for fifteen days, with periodic stirring and shaking. The resulting mixture was filtrated. A rotary evaporator was used for concentrating the mixture at 60°C. 37g crude extract was obtained and then underwent a stepwise fractionation process depending on polarity properties. The procedure commenced with n-hexane and concluded with dichloromethane, utilizing 300 ml of each solvent. The fractions were subsequently filtered with Whatman filter paper. It was concentrated with a rotary evaporator at 60°C. The collected fractions were thereafter kept at 4°C for further analysis.

Phytochemical study of the fractions:

The solvent fractions were subjected to phytochemical analysis including alkaloids, glycosides, phytosterols, carbohydrates, proteins, saponins, flavonoids, tannins, phenols, terpenes, lipids and fixed oils, utilizing standardized methodologies. [13,14]

Experimental animals

Female Swiss Albino Mice (25-35g) were sourced from the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B) Dhaka animal resources facility. The mice were kept in hygienic, climate-controlled cages, maintaining a light-dark cycle of12-hour at a temperature of 25±2°C within the animal facility. The subjects received a standard laboratory diet and had *ad libitum* water. A 3–4-day acclimatization period occurred prior to the starting of the experiments, during which the animals were kept without food for 12 hours, because recent food intake can interfere with the hematological parameters. Approval for animal handling for this research was granted by the Animal Ethics Review Committee (AERB) of the Faculty of Pharmaceutical Sciences at the University of Science and Technology Chittagong(Reg. no. USTC/AEAC/24/025).

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Assessment of analgesic potential:

Peripheral analgesic activity utilizing the induced writhing

The study utilized the Koster method, as redesigned by Dambisya and Lee.[15]Group 1 denoted as the control group. Group 2 functioned as the control group. Groups 3 and 4 served as the experimental groups, receiving200and 400 mg/kg of NHS(n-hexane extract of seed) treatment, respectively. Groups 4 and 5 were treated with the test doses of DMS at 200 and 400 mg/kg. 0.7% glacial acetic acid Intraperitoneal injection was utilized for inducing writhing via at a dosage of 10 ml/kg. [16]The animals of standard group underwent pretreatment fifteen minutes prior to acetic acid administration through intraperitoneal route, whereas the test groups received pretreatment thirty minutes before the acetic acid was administered. Following the acetic acid administration, the frequency of writhing in each mouse was recorded over a 20-minute period, commencing 5 minutes post-administration. The abdominal writhing inhibition percentage was assessed using a specific formula: [17,18]

$$\% \text{ Of inhibition} = \frac{Nc - Nt}{Nc} \times 100$$

Nc = number of writhing observed in the control group.
Nt = count of writhing occurrences in the experimental groups.

Paw-licking model

The paw-licking test induced by formalin was conducted using the scientifically described method.[19] Group 1, denoted as the control, Group 2, the standard, Group 3 and 4 denoted as the test groups and were administered 200and 400mg/kg of NHS. Group 4 and 5marked as 200and 400mg/kg of DMS test groups. To elicit a biphasic pain response, 1% formalin 20 µl was subcutaneously injected into the mice right hind paw, 60 minutes following the plant extracts administration and the standard treatment. The duration of licking and biting of the injected paw was measured as a metric for pain response. Responses were assessed in two distinct phases: the early phase (zero to five minutes, neurogenic) and the late phase (fifteen to thirty minutes post-formalin injection). The pain inhibition percentage was determined using the following formula:[8]

$$\text{Pain inhibition (\%)} = \frac{\text{Reaction time (Control)} - \text{Reaction time (Treatment)}}{\text{Reaction time (Control)}} \times 100$$

Antioxidant potential assessment

In-vitro DPPH Radical Scavenging assay:

NHS and DMS were examined for determining their reducing power by observing their ability to scavenge radicals using the DPPH method.[20] The DPPH(2,2-diphenyl-1-picrylhydrazyl) solution changes its color from violet to pale yellow when it interacts with a reducing agent. Different concentrations of the fractions were involved in this assay. 5 mg of NHS and DMS reactions were individually dissolved in 10 ml of methanol in test tubes. Stock solutions(500 µg/ml)underwent serial dilution to achieve concentrations of 250, 125, 62.5, 31.25, and 15.63 µg/ml using methanol, followed by room temperature incubation for three minutes. The mixture was vigorously agitated, and the microplate reader recorded the absorbance of the residual DPPH at an absorbance of 517 nm after 30 minutes. The DPPH radical scavenging rate was calculated utilizing the following equation:[17]

$$\% \text{ of Scavenging effect (DPPH)} = \left[\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}}\right] \times 100$$

In- vitro Hydrogen Peroxide Scavenging assay

The capacity of NHS and DMS to scavenge hydrogen peroxide was evaluated using a previously established method.[21]Hydrogen peroxide was prepared in a phosphate buffer solution at pH 7.4. 5 ml of NHS and DMS extracts were separately dissolved in 10 ml of distilled water in test tubes. The solutions are stock solutions with a concentration of 500 µg/ml. Stock solutions were serially diluted to concentrations of 250, 125, 62.5, 31.25, and 15.63 µg/ml using distilled water. The absorbance was measured 10 min later at 230 nm in comparison to a blank solution which contains phosphate buffer in absence of hydrogen peroxide.[22]

The calculation for scavenging hydrogen peroxide abilities was calculated utilizing the following equation:[23]

$$\% \text{ of scavenging effect } (H_2O_2) = \left[\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{absorbance of control}}\right] \times 100$$

Anti-inflammatory potential assessment

Formalin induced Paw edema

Paw edema induced by formalin is a highly appropriate test method for assessing sub-acute anti-inflammatory effects. The injection of formalin triggers edema and a rise in vascular permeability. [24]Group 1, group 2 served as control and standard respectively. Group 3 and

Group 4 were the test groups and were given 200mg/kg and 400mg/kg of NHS and DMS. Paw edema was instigated by subcutaneously injecting 50µl of 5% formalin one hour after the treatment was administered and 30 min following the standard treatment. Circumference of paw (in mm) was measured with slide calipers prior to and following formalin injection, and the percentage of paw edema inhibition was subsequently calculated:

$$\text{Inhibition (\%)} = \frac{(Ct-C_0)control-(Ct-C_0)treated}{(Ct-C_0)control} \times 100$$

Ct = Average circumference of paw for each group at various time intervals
C_0 = Average circumference of paw for each group prior to formalin injection

Carrageenan induced Mice paw edema
Inducing acute inflammation with carrageenan is an effective method for evaluating the anti-inflammatory potential of drugs. [25]The experiment utilized the use of carrageenan as an inflammatory agent to cause edema in the right hind limb of mice.[26]Group 1 and Group 2 was given the control and standard treatment. Group 3 and Group 4 were the test groups and were given 200mg/kg and 400mg/kg of NHS, respectively. 1 hour post administering the treatment sample and 30 min following the standard dose, a 100 µl carrageenan subcutaneous injection was administered into the right hind paw. Circumference of paw (in mm) was measured prior to carrageenan administration and subsequently at the conclusion of the 1st to 4th hours. The inhibition percentage of edema was then subsequently analyzed using the following formula:

$$\text{Percentage of inhibition} = \frac{(Ct-C_0)control-(Ct-C_0)treated}{(Ct-C_0)control} \times 100$$

Ct = Average circumference of paw for each group at various time intervals
C_0 = Average circumference of paw for each group prior to formalin injection

Analysis of Statistics
The scientific software SPSS, version 16.0was utilized to look at the analysis as mean ± SEM (Standard Error Mean). When compared to the control, results below *p<0.05, **p<0.01, or ***p<0.001 were thought to be significant statistically. Microsoft Excel 2013 was used to make the graphs.

Results and Discussion

Phytochemical assessment:
Citrus maxima seed fractions contained alkaloids, glycosides, resin, fats and fixed oils, tannins, flavonoids, steroids, alkaloids, glycosides, terpenoids, etc (Table 1).

Table 1: Phytochemical Constituents of fractions of *Citrus maxima* seed.

Phytochemicals	MES	NHS	DMS	Remarks
Terpenoids				+
Flavonoids				+
Saponins				-
Phenols	MES	NHS	DMS	-
Tannin				+
Phlobatanins				-
Steroids				-
Anthraquinone				-
Alkaloids				+
Glycosides				+
Resin				+
Carbohydrates				-
Proteins				-
Fats and fixed oils				+

Analgesic activity
The present research investigated the analgesic properties of the obtained fractions from *Citrus maxima* methanolic extract using two established nociceptive models the writhing reflex induced by acetic acid and the paw licking test induced with formalin. The results indicated that both the treatments, methanolic extract (MES)[8] and the n-hexane soluble fraction (NHS) exhibited significant analgesic activity, demonstrating potential as peripheral and central anti-nociceptive agents respectively. The writhing test induced through acetic acid is recognized as an effective chemical model of pain.[27]Prostaglandins (PG) are associated with hyperalgesia by altering the transduction capabilities of free nerve endings, enabling stimuli that typically don't provoke pain to do so.[28]Raised prostaglandins levels, especially PGE2 and PGF2α,[29]along with lipoxygenase derivatives from leukotriene synthesis,[30]have been detected in the peritoneal fluid following acetic acid injection intraperitoneally[31]leading to inflammatory pain characterized by hind limb extension and abdominal muscle contraction in mice. In the acetic acid-induced writhing test, MES exhibited the most significant writhing inhibition at 400 mg/kg (57.49%,P<0.001),[8] surpassing the effects of the reference standard, Morphine, 58.94% inhibition (P<0.001), while NHS displayed comparable results, particularly at higher doses, 52.66% inhibition(P<0.01) (Table 2).This indicates that these extracts may influence pain pathways, probably by inhibiting pain mediators such prostaglandins and leukotrienes. The formalin-induced paw licking assay (Table 3) further corroborated the analgesic effectiveness of these extracts. The analyzed effect may result from the suppression of the excretion of several mediators, including histamine, serotonin, and kinins. The formalin induced test is a recognized and viable technique for investigating central sensitization phenomena at the spinal level following a peripheral inflammatory condition[32].MES and NHS showed a significant and very high potential to reduce the formalin-induced pain during the late phase as a peripherally acting anti-nociceptive agent at both doses. The potential was more in the high dose. MES and NHS provided significant pain relief in both the early and late phases, indicating their potential to address both acute and inflammatory pain. In contrast, DMS exhibited moderate analgesic activity, highlighting the variability in efficacy among different fractions, 36.71% inhibition (P<0.01). These findings corresponds with prior research indicating the analgesic and Anti-inflammatory properties of phytochemicals present in *Citrus maxima* methanol extract of seeds, specifically flavonoids and tannins.[8] Based on the findings, it seems that these chemicals may be responsible for the analgesic effects.

Antioxidant potential assessment
Oxidative stress is a primary contributor to neurodegenerative illnesses, perhaps attributable to the elevated levels of polyunsaturated

fatty acids in the brain.[33,34]Consequently, antioxidants might significantly aid in the management of neurodegenerative disorders due to the brain's substantial consumption of metabolic oxygen, which tends to increase the synthesis of reactive oxygen species (ROS). Reactive oxygen species (ROS) in the brain cause lipid oxidation due to the raised concentration of phospholipids, that are readily susceptible to free radical attack, resulting in a progressive deterioration of neuronal and cognitive processes.

Table 2: Screening and comparison of analgesic effect of crude methanol extract of *Citrus maxima* seeds with its solvent fractions by calculating mean writhing of mice and percentage (%) inhibition of writhing using writhing method by inducing Acetic acid.

Group	Writhing Number (Mean ± SEM)	% of Writhing Inhibition
Control	41.40±1.08	0
Standard	17.00±0.71***	58.94
MES200	21.00±0.55***	49.28
MES 400	17.60±0.93***	57.49
NHS 200	22.80±0.97***	44.93
NHS 400	19.60±2.29**	52.66
DMS 200	32.20±0.66**	22.22
DMS 400	26.20±1.85**	36.71

MES = Methanol extract of seed, NHS = n-hexane fraction of seed, DMS = Dichloromethane fraction of seed. Results are exhibited as mean ± SEM (N= 5), *P< 0.05, **P<0.01, ***P<0.001 were considered as Statistically significant when comparing experimental values to the control group.

Table 3: Screening of analgesic potentials of crude methanol extract of *Citrus maxima* seed and it's solvent fractions by calculating mean paw licking time and percentage (%) inhibition of pain at different time using paw licking test inducing Formalin.

Group	Dose (mg/kg)	0-5 minute(Early Phase)		15-30minute(Late phase)	
		Paw licking time(sec)	Pain Inhibition (%)	Paw licking time(sec)	Pain Inhibition(%)
Control	10	52.88±4.49	-	73.00±4.87	-
Standard	10	8.60±0.69**	83.74	2.12±0.45***	97.09
MES	200	10.36±4.11**	80.41	2.71±0.82***	96.29
MES	400	9.19±4.25**	82.62	2.33±1.08***	96.81
NHS	200	11.06±5.32**	79.08	4.45±3.95***	93.90
NHS	400	10.596±3.72**	79.96	2.94±2.23***	95.97
DMS	200	13.26±1.93**	74.92	5.97±1.25***	91..82
DMS	400	12.19±1.04**	76.95	5.47±1.42***	92.51

MES = Methanol extract of seed, NHS = n-hexane fraction of seed, DMS = Dichloromethane fraction of seed. Results are exhibited as mean ± SEM (N= 5), *P< 0.05, **P<0.01, ***P<0.001 were considered as significant Statistically when comparing experimental values to the control group.

The antioxidant efficacy assessment of *Citrus maxima* seed extract and its solvent fractions involves DPPH radical and Hydrogen Peroxide scavenging assay. In present investigation, the antioxidant properties of the fractions were evaluated using the DPPH assay. The study revealed that the reference standard ascorbic acid (AA) displayed a 96.014% scavenging effect (Table 4) at the highest concentration, with an IC_{50} value of 0.534µg/ml, demonstrating its highly effective free radical scavenging abilities. In contrast, the negative control showed no antioxidant activity. The extract MES[12](Table 5) and fraction NHS (Table 6) exhibited milder and concentration-dependent free radical scavenging effects compared to the reference standard. Additionally, DMS demonstrated potent antioxidant effects at higher concentrations (Table 7), with a much lower IC_{50} value compared to MES and NHS. However, it did not exceed the scavenging effect percentage of AA and did not show significant potentials at lower concentrations in comparison to the reference standard (Figure 1).

Table 4: Antioxidant potential of the investigated extracts of *Citrus maxima* seeds fraction through DPPH scavenging assay.

Group	Equation	R^2	IC_{50}
Standard	y = 16.235x + 54.429	0.9623	0.534
MES	y = 5.0077x + 35.036	0.6874	973.191
NHS	y = 5.2524x + 35.927	0.9474	477.911
DMS	y = 12.541x + 24.925	0.8724	99.872

Table 5: Antioxidant potential of the investigated extracts of *Citrus maxima* seeds fraction through Hydrogen Peroxide scavenging assay.

Group	Equation	R^2	IC_{50}
Standard	y = 14.516x + 49.898	0.9941	1.016
MES	y = 19.3x - 10.674	0.9866	1392.29
NHS	y = 26.241x - 23.486	0.9907	631.58
DMS	y = 25.929x + 9.0537	0.9806	37.946

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Table 6: Screening and comparison of anti-inflammatory potentials of crude methanol extracts (MES) with n-hexane (NHS) and Dichloromethane (DMS) soluble fractions of *Citrus maxima* seeds by calculating mean paw circumference and % inhibition of edema utilizing mice paw edema test inducing Formalin.

Group	Dose (mg/kg)	Pre-injection mean paw circumference (mm)	Post-injection mean paw circumference (mm) (% of edema inhibition) 1 hr	2 hr	3 hr	4 hr
Control	10	8.10±0.33	14.40±0.40 -	14.20±0.37 -	13.80±0.20 -	13.60±0.29 -
Standard	10	8.90±0.24	11.80±0.34** 53.97%	11.30±0.34** 60.66%	9.90±0.19*** 82.46%	9.40±0.19*** 90.91%
MES	200	7.60±0.19	12.00±0.27** 30.16%	11.40±0.29** 37.70%	10.80±0.25*** 43.86%	10.10±0.24*** 54.55%
MES	400	7.20±0.25	11.00±0.57** 39.68%	10.40±0.53** 47.54%	9.60±0.40*** 57.89%	9.30±0.41*** 61.82%
NHS	200	8.40±0.37	12.00±0.32* 42.86%	11.20±0.25** 54.10%	10.20±0.37*** 68.42%	9.40±0.29*** 81.82%
NHS	400	8.50±0.27	11.40±0.51* 53.97%	10.70±0.41** 63.93%	9.90±0.29*** 75.44%	9.20±0.34*** 87.27%
DMS	200	7.80±0.25	11.90±0.19*** 34.92%	11.30±0.12*** 42.62%	10.60±0.19*** 50.88%	10.10±0.19*** 58.18%
DMS	400	8.40±0.43	12.40±0.29** 36.51%	11.80±0.25*** 44.26%	11.00±0.35*** 54.39%	9.60±0.19*** 78.18%

Table 7: Screening and comparison of Antiinflammatory potentials of crude methanol extracts (MES) with n-hexane (NHS) and Dichloromethane (DMS) soluble fractions of *Citrus maxima* seeds by calculating mean paw circumference and % inhibition of edema utilizing Carrageenan-induced mice paw edema test.

Group	Dose (mg/kg)	Pre-injection mean paw circumference (mm)	Post-injection mean paw circumference (mm) (% of edema inhibition) 1 hour	2 hour	3 hour	4 hour
Control	10	9.80±0.58	14.60±0.51 -	15.20±0.49 -	14.80±0.37 -	14.20±0.49 -
Standard	10	10.20±0.37	13.50±0.35 31.25%	13.00±0.35 48.15%	11.20±0.41** 76%	10.80±0.44** 86.36%
MES	200	9.60±0.51	13.90±0.33 10.41%	13.40±0.33 29.63%	12.40±0.29** 44%	11.70±0.30** 52.27%
MES	400	9.30±0.37	13.30±0.37 16.67%	12.80±0.37 35.19%	11.70±0.44** 52%	11.20±0.34** 56.82%
NHS	200	9.40±0.29	13.10±0.33 22.92%	12.30±0.34* 46.30%	11.60±0.29** 56%	11.00±0.27** 63.64%
NHS	400	9.50±0.45	12.90±0.51 29.17%	12.20±0.51* 50%	11.40±0.53** 62%	10.90±0.43** 68.18%
DMS	200	8.80±0.25	12.90±0.33 14.58%	12.50±0.31** 31.48%	11.90±0.33*** 38.00%	11.30±0.33*** 43.18%
DMS	400	9.30±0.25	13.10±0.24 20.83%	12.40±0.29** 42.59%	11.80±0.20*** 50.00%	11.10±0.24*** 59.09%

MES = Methanol extract of seed, NHS = n-hexane fraction of seed, DMS = Dichloromethane fraction of seed. Results are exhibited as mean ± SEM (N= 5), *P< 0.05, **P<0.01, ***P<0.001 were considered as significant Statistically when comparing experimental values to the control group.

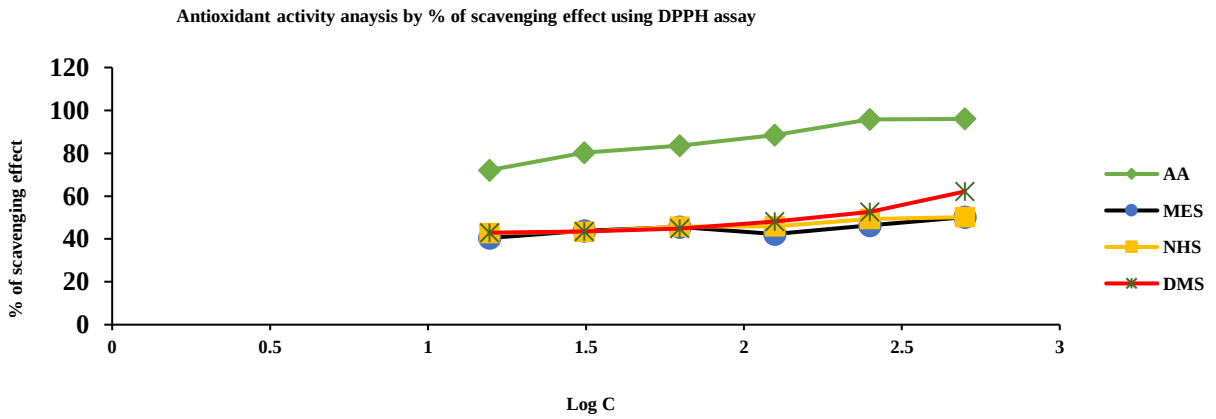

Figure 1: Graphical representation of different concentration of Crude methanol extract of *Citrus maxima* seeds, its solvent fractions by comparing % of scavenging effect using DPPH free radical scavenging assay.

Hydrogen peroxide can enter the body as vapor or mist, and through contact with the eyes or skin.[35] When hydrogen peroxide breaks down into water and oxygen, it can create hydroxyl radicals which may harm the body's DNA by initiating lipid peroxidation. Ascorbic acid (AA), the reference standard demonstrated the highest H_2O_2 scavenging effect of 96.014% at the highest concentration. It effectively eliminated H_2O_2 even at the lowest concentration, while the control showed no scavenging effect. MES[12] and NHS showed a mild H_2O_2 scavenging effect compared to AA at high concentrations, but they were not effective as antioxidants at low concentrations. The IC_{50} value of these extracts was much higher than that of AA. DMS was highly effective at scavenging H_2O_2 at high concentrations compared to AA, but its ability to eliminate H_2O_2 moderately decreased as the concentration decreased. The IC_{50} value of DMS was much lower than that of MES and NHS. All effects were dependent on the concentration (Figure 2).

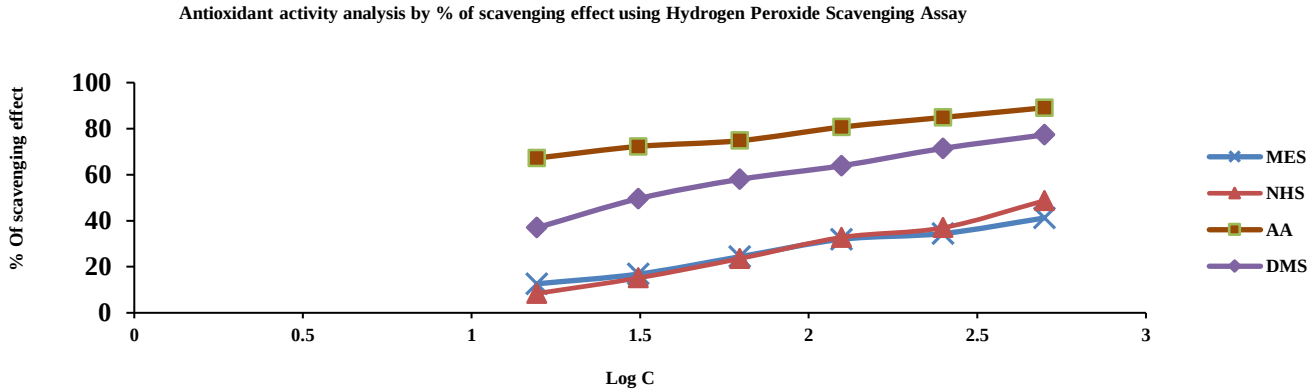

Figure 2: Graphical representation of scavenging effect using Hydrogen Peroxide scavenging assay of different concentration of Crude methanol extract of *Citrus maxima* seeds, its solvent fractions.

Anti-inflammatory potential assessment

Pain and inflammation are closely related, as inflammation often triggers pain as part of the body's defense mechanism.[36]Inflammatory cells release various substances, including cytokines, prostaglandins, and bradykinin, which sensitize nerve endings and increase pain perception.[37] The effects of different treatments on paw circumference and edema inhibition were assessed at various time points of post-injection following the formalin-induced and Carrageenan-induced paw edema method. In the formalin induced paw edema test method, the Standard group(Indomethacin)proved to be highly efficacious in reducing edema, 90.91% inhibition (***P<0.001) by 4 hours. Among the experimental groups, MES extract at both 200 and 400 mg/kg showed lesser efficacy, with 54.55% inhibition (p<0.05*) at 4 hours for the higher dose(400 mg/kg). The NHS treated fraction group yielded significant results, particularly at the higher dose of 400 mg/kg, achieving 87.27% inhibition (p<0.05*) at 4 hours. This marked reduction signifies the potential of this treatment to effectively manage edema. In contrast, the dichloromethane fraction (DMS) group demonstrated moderate efficacy, with the 400 mg/kg dose leading to 78.18% inhibition (p<0.05*) at the same time point. In the Carrageenan induced model, the Standard treatment showed notable reductions in paw circumference, achieving 86.36% edema inhibition (p<0.01**) at 4 hours post-treatment. The MES at 200 and 400 mg/kg displayed moderate Anti-inflammatory effects, with the higher dosage resulting in 56.82% (**P<0.01) inhibition at the 4-hour mark. The NHS fraction also demonstrated moderately significant activity, with the 400 mg/kg group achieving 68.18% (**P<0.01) inhibition. The DMS extracts exhibited less significant effects, with the 400 mg/kg dosage reaching 59.09% (**P<0.01) inhibition.

Conclusion

Results showed thatNHS and DMS, fractions isolated from *C. maxima* seed methanol extract, had dose-dependent analgesic and anti-inflammatory effects, but showed little evidence of free radical scavenging activity. These findings create a scientific base for folk medicinal use. Future investigation is recommended for isolating the polar and non-polar compounds responsible for the biological activities.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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