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Investigating the Multifaceted Therapeutic Potential of *Firmiana colorata* (Roxb.) R.Br. Leaf Extract: A Natural Remedy to Pain, Fever, and Oxidative Stress

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ABSTRACT

Firmiana colorata (Roxb.), commonly found in the tropical and subtropical regions of South Asia, is one such plant widely used in Bangladeshi folk medicine. The present study aims to scientifically investigate the analgesic, antipyretic, and antioxidant activities of *F. colorata* through in vivo and in vitro assays, using crude methanol leaf extract and its various solvent fractions. Qualitative screening of phytochemicals of the plant's methanol extract was done using standard procedure. Utilizing the writhing model induced by the acetic-acid and paw-licking model triggered by formalin, the analgesic activity was assessed. Applying brewer's yeast triggered pyrexia method the antipyretic effect was investigated. Potential against oxidative stress was examined by DPPH free radical and H_2O_2 scavenging assays. The writhing test showed significant analgesia, with NHFC extract (400 mg/kg) exhibiting the strongest inhibition of writhing 81.78%, indicating strong peripheral analgesia. The paw-licking test revealed mild central analgesic effects during the early phase for all extracts. Regarding antipyretic activity, all extracts significantly $p<0.001$ reduced the raised body temperature in a brewer's yeast-induced hyperpyrexia model, with NHFC showing the most promise as a natural alternative to conventional antipyretics. DMFC with an IC_{50} value of 34.67 µg/ml showed the most potent DPPH free radical neutralizing action among the extracts. It is believed that phytocomponds such as alkaloids, flavonoids, and tannins contribute to these therapeutic effects. The study offers insightful information on the medicinal potential of *F. colorata*, supporting its traditional uses and encouraging further exploration of its bioactive compounds for potential health benefits.

Keywords: *Firmiana colorata*, Phytochemicals, Analgesic, Antipyretic, Antioxidant, Fraction, N-hexane, Dichloromethane.

Introduction

Natural treatments have long been a favorite among people because of their remarkable therapeutic qualities, which can treat complicated medical conditions with little to no side effects and at a minimal cost.[1] Terpenoids, flavonoids, phenols, saponins, tannins, and many more secondary metabolites have been scientifically attributed to the pharmacological properties of medicinal plants.[2] There is evidence-based research conducted all around the world to confirm the benefits of medicinal plants, and some of these studies have shed light on the synthesis of phytochemicals derived from a plant that has therapeutic properties.[2] *Firmiana colorata* (Roxb.), commonly known as Udal in Bangladesh, has garnered attention not only for its remarkable beauty but also for its significant ecological and potential medicinal contributions.[3]

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This deciduous tree, native to East Asia, is characterized by its large, heart-shaped leaves and striking flowers, making it a favored choice in urban landscaping and ecological restoration projects.[4].Locals are well aware of this species' traditional therapeutic usefulness, which includes the ability to treat cholera and jaundice. When intestinal dysfunction arises, *Firmiana colorata* leaf juice is blended with *Duabanga grandiflora* leaf juice, *Chrysopogon aciculatus* leaf juice, *Alpinia nigra* rhizome juice, *Hyptis suaveolens* leaf juice, *Nigella sativa* seeds, and the concoction is taken orally.[3] Research on the flora that the Bhilla tribe of Maharashtra uses for traditional treatment. There, *Firmiana colorata* seeds are used to cure general weakness.[5] Identification of plants with anticancer and antibacterial properties through an ethnobotany of medicinal plants in Andhra Pradesh's Eastern Ghats found that *Firmiana colorata*'s tender leaf juice works well to prevent wound and eye infections.[6] In recent years, however, researchers have begun to delve deeper into its medicinal properties, particularly its antimicrobial, anti-inflammatory, and antioxidant activities.[7] Historically, various cultures have utilized different parts of *F. colorata* for their health benefits, indicating a long-standing recognition of its potential therapeutic effects. These traditional uses hint that the plant has beneficial bioactive chemicals that could offer significant health advantages.[3] As modern medicine increasingly explores natural alternatives to conventional pharmaceuticals, the analgesic and antipyretic properties of *F. colorata* have gained particular relevance.[8] With growing concerns over the side effects associated with standard painkillers and fever reducers, the search for effective, plant-based alternatives has never been more pressing.[9] Moreover, a significant factor in the emergence of numerous health conditions, encompassing chronic inflammation, neurological illness, and cardiovascular diseases is oxidative stress. Cellular damage may result from the body's imbalance between antioxidants and free radicals, making the antioxidant capacity of plant-derived compounds vital in combating

these harmful effects.[10] By harnessing the natural properties of plants like *F. colorata* we can potentially mitigate oxidative stress and promote overall health and well-being. This research aims to systematically evaluate the analgesic, antipyretic, and antioxidant qualities of crude methanol leaf extract of *F.colorata* and its solvent fraction.

By employing a combination of laboratory assays and in vivo animal studies, this investigation will pinpoint the bioactive agents in charge of these therapeutic effects and investigate the underlying mechanisms of action. Through this comprehensive assessment, the study seeks to enhance the understanding of the medicinal potential of *F. colorata*, contributing valuable insights to the growing body of knowledge on traditional medicinal plants. Ultimately, this research aims to encourage further investigation into the beneficial compounds of *F. colorata*, paving the way for new, natural approaches to health care and wellness.

Materials and Methods

Solvents and chemicals

Square Pharmaceuticals Ltd. (Gazipur, Bangladesh) supplied the Diclofenac sodium and Paracetamol. We purchased Morphine sulfate from Renata Ltd. (Mirpur, Bangladesh). Sigma-Aldrich (Humburg, Germany) supplied Methanol, n-hexane, dichloromethane, 2, 2-Diphenyl-1-picryl-hydrazyl (DPPH). Acetic acid, formalin, ascorbic acid, and phosphate buffer saline were sourced from local vendors. Every chemical compound and reagent utilized in this investigation was of analytical grade.

Collection and utilization of plant leaves for extraction

The leaves of *Firmiana colorata* were gathered from hilly regions of the Chattogram Division (21.670°N latitude and 92.090°E longitude) of Bangladesh in November 2019. A renowned taxonomist ensured the authentication of the plant as *Firmiana colorata* and the specimen was preserved as MK 070919-422. After being cleaned of filth with running tap water the collected leaves were chopped into tiny fragments and allowed to dry for a week in a semi-shady area. A high-capacity grinder from the University of Chittagong's pharmacy department's phytochemical research laboratory was used to grind the plant materials into a coarse powder after drying. After that, the powdered ingredients were put into an airtight container. Four (4) liters of pure methanol were used to soak 700 grams of the powdered plant leaf in a sterile 5-liter flask with a round bottom. After being covered with foil, the container containing the content was stored for 15 days while being shaken and stirred sporadically. After 15 days the solution was filtered, and a rotary evaporator (Stuart, UK) at 60°C temperature was used to concentrate the filtrate. The weight of the resulting crude methanol leaf extract (MEFC) was 15 grams. For further studies, the yield was kept in a beaker sealed with foil at 4°C in the refrigerator. The extract's yield percentage (%) was calculated by using the below formula.[11]

$$\%\ of\ yield\ extract = \frac{Weight\ of\ extracted\ material}{Weight\ of\ \ crude\ powder} \times 100 \quad (1)$$

The crude methanol leaf extract of *Firmiana colorata* had a yield of 2.14%.

Solvent–solvent partitioning

Solvent-solvent partitioning of the crude methanol extract, using n-hexane and dichloromethane as successive solvents, was carried out in accordance with the approach introduced by Kupchan and Tsou and revised by Van Wagenen. [12, 13]

Qualitative phytochemical study of the extract

Phytochemical screening of crude methanol leaf extract was done, to find out the presence of phytochemicals such as alkaloids, anthraquinone glycosides, cardiac glycosides, resins, carbohydrates, steroids, proteins, terpenoids, tannins, flavonoids, phenol, fat, and oil and saponins by employing conventional technique.[1]

Experimental animals

Weighing between 25-35 grams, male Swiss albino mice were gathered from ICDDRB, Dhaka. In the animal facility, the mice were housed in hygienic, dry propylene cages where 25±2°C temperature, 60-70% relative humidity, and a 12-hour light-dark cycle were maintained. The mice received a typical diet in the lab. The subjects were prevented from taking food before 12 hours of conducting the trial. The animal ethics review committee (AERB) of the University of Science and Technology Chittagong's faculty of pharmaceutical sciences approved this trial under the number of USTC/AEAC/24/030 and all the parts related to animal research adhere to ARRIVE Guidelines.

Study design for in vivo testing

To assess the analgesic activity using two different methods, 8 groups of mice were taken for each method; each group consisted of 5 mice. Group (I) was denoted as the control group and was given a solution of 1% Tween 80 orally; in the writhing test Diclofenac sodium (50 mg/kg) was given orally and in paw licking test morphine sulfate (10 mg/kg) was given at oral route to Group (II) that served as a standard group. Group (III), Group (IV), Group (V), Group (VI), Group (VII), and Group (VIII) served as experimental groups. Group (III), Group (IV) received varying doses (200 and 400 mg/kg respectively) of methanol leaf extract of *F.colorata*, Group (V), Group (VI) received varying doses of n-hexane fractions (200 and 400 mg/kg respectively) and Group (VII) and Group (VIII) received varying doses of dichloromethane fractions (200 and 400 mg/kg respectively). For the pyrexia test induced by brewer's yeast, 40 mice were chosen randomly and split up into 8 groups of five mice each. Group (I) was control, Group (II) received conventional treatment (Paracetamol 150 mg/kg) as standard and the remaining 6 groups (Group III to Group VIII) received crude methanol leaf extract of *F.colorata*, n-hexane, and dichloromethane fractions at doses of 200 and 400 mg/kg, respectively.

Evaluation of analgesic capacity

Acetic acid triggered writhing test

The plant extract's ability to relieve pain was investigated by using the Koster method as amended by Dambisya and Lee.[14, 15] This method assessed the analgesic behavior in mice resulting from a noxious stimulation. In this test, pain sensation is triggered in animals through the administration of acetic acid via the abdomen, and as a result, animal bodies contract periodically to get away from pain. "Writhing" is the phrase used to describe the animal's constant contraction or squirming. Animals in the standard group had a pretreatment 15 minutes before the administration of acetic acid, while test groups received a pretreatment 30 minutes prior. 5 minutes after acetic acid was administrated, each mouse's writhing was counted over 20 minutes. The equation below was adopted to generate the amount of writhing suppression, which gauged the level of analgesia.

$$\%\ inhibition\ of\ pain = (Wc - Wt)/Wc \times 100 \quad (1)$$

Here, Wc = Number of writhing in the control group, and
Wt = Number of writhing in the treatment group

Paw licking method

The paw licking test was used to examine the pain-relieving effects of the examined extracts and it's a persistent-pain model described by Hunskaar and Hole.[16] 60 minutes after administering control, standard, and test sample orally; pain was triggered by a subcutaneous injection of formalin (1%, 20 µl) to each mouse on their the right back paw. The length of time the mice spent biting or licking their paw was taken as a reflection of the pain reaction. The reactions were observed for the initial 5 min (early phase, neurogenic) and later, at 15-30 min (late phase, inflammatory).[17] The formula below (equation 2) was employed to calculate the pain mitigation percentage (%).

$$\text{Pain suppression } (\%) = \frac{Reaction\ time\ (Control) - Reaction\ time\ (Treatment)}{Reaction\ time\ (Control)} \times 100 \quad (2)$$

Evaluation of antipyretic capacity

A method described by Adams et al. of inducing pyrexia with brewer's yeast was used to measure the antipyretic effect.[18] Before the study started, a digital thermometer was used to record the mice's rectal temperature. Just below the nape of their necks, mice subcutaneously

received an injection of 15% brewer's yeast (aqueous suspension 10 ml/kg) followed by a mild massage to induce hyperthermia. To evaluate pyretic response to the yeast, pre-drug temperature was recorded 24 hours after the injection. Animals whose body temperature had been raised by at least 1 °F were used. Temperature readings were gathered one, two, three, and four hours later following the drug treatment. The percentage (%) decrease in rectal temperature was computed by the following formula (equation 3).[19]

$$Reduction\ of\ pyrexia(\%) = \frac{B-C}{B-A} \times 100 \quad (3)$$

Here, A= Mice's typical body temperature, B= rectal temperature of mice, 24 h post-treatment with brewer's yeast, and C= Rectal temperature of mice, after receiving test samples at a varying time point.

Assessment of in vitro antioxidant potential
Scavenging assay using DPPH free radical
The free radical scavenging capacity of several *F.colorata* extracts was determined using stable DPPH (2, 2-diphenyl-1-picrylhydrazil) in comparison to ascorbic acid according to the established procedure with a few minor adjustments.[20] In 2ml of varying concentrations of either test sample or standard (15.63, 31.25, 62.5,125, 250, and 500 µg/ml), a 3 ml of 0.004% DPPH solution was dissolved and kept in a dark room for 30mins at room temperature. DPPH solution was used as a control which was prepared by using the method of sample preparation but without the sample. Methanol was taken as blank and after 30 minutes using a UV-visible spectrophotometer (Halo SB-10, Indonesia), at a wavelength of 517 nm the absorbance was taken. The DPPH radical neutralizing capacity was calculated as the inhibition percentage (%) using the following formula (equation 4),

$$\%\ inhibition\ of\ DPPH\ scavenging\ activity = \frac{A0-A1}{A0} \times 100 \quad (4)$$

Where, A_0 = Absorbance of control (freshly prepared DPPH solution) and A_1 = Absorbance of test sample/standard.

Hydrogen peroxide radical scavenging assay
A previously reported approach has been utilized to evaluate the test sample's capacity to neutralize H_2O_2.[21] Various concentrations (15.63, 31.25, 62.5,125, 250, and 500 µg/ml) of 1 ml of ascorbic acid solution as a standard and sample solution were taken and 0.6 ml of H_2O_2 solution was added to each. After 10 minutes using a UV-Vis spectrophotometer (Halo SB-10, Indonesia) absorbance was taken at a wavelength of 230 nm, in comparison to a blank solution filled with phosphate buffer and no H_2O_2. The solution that served as a control was a mix of 1 ml of phosphate buffer and 0.6 ml of H_2O_2. The abilities to scavenge the H_2O_2 were calculated utilizing the below formula (equation 5),

$$\%\ inhibition\ of\ hydrogen\ peroxide\ radicals = \frac{A0-At}{A0} \times 100 \quad (5)$$

Where, A_0 = Absorbance of control and A_1 = Absorbance of test sample/standard.

Determination of median inhibitory concentration (IC_{50})
The concentration of the sample needed to neutralize 50% of DPPH or H_2O_2 after a specific exposure duration is the median inhibitory concentration (IC_{50}). Using the linear regression method, by plotting the percent scavenging effect versus the corresponding logarithm of concentration the IC_{50} was determined. Trend line fit linear regression analysis was used to convert the concentration-scavenging data into a straight line (Microsoft Excel, 2013). The graph paper showed an approximate linear association, and the best-fit line was used to calculate IC_{50}.

Statistical analysis
To express all the obtained data the mean ± SEM (Standard error of Mean) was used. The data analysis tool "Statistical Package for Social Science" (SPSS, 16.0) was used to analyze the data statistically by one-way ANOVA and a post hoc Dunnett's test. Where data below *p<0.05, **p<0.01, and ***p<0.001 in relation to control were considered to be statistically significant. Microsoft Excel 2013 was used to obtain IC_{50} values by using linear regression models.

Results and Discussion

Locals are familiar with the plant under investigation, *F. colorata*, due to its several traditional uses. Previous studies have revealed a few phytochemicals and various in vitro pharmacological effects in methanol extract, which led to the current investigation to evaluate the effectiveness of methanol leaf extract and its solvent fraction as analgesic and antipyretic agents in animal model and as antioxidants.[7]

Phytochemical analysis
Several phytoconstituents were identified by the initial phytochemical analyses performed on the plant's methanol leaf extracts; Table 1 includes them.

Table 1: Phytochemicals' presence in *F.colorata* methanol leaf extract

Phytochemicals	Results
Alkaloids	+
Flavonoids	+
Phenols and Tannins	+
Glycosides	+
Cardiac Glycosides	+
Anthraquinone Glycosides	+
Saponins	-
Carbohydrates	+
Proteins	-
Fats And Oils	+
Steroids	-
Terpenoids	+
Resins	+

Note: "+" indicates phytochemicals' presence and "–" indicates phytochemicals' absence.

Firmiana colorata extract's in vivo investigation
Analgesic potential analysis
Writhing model Mice that experience acetic acid-induced discomfort respond by contracting their bellies and stretching their hind limbs. From tissue phospholipids, free arachidonic acid is released. A localized inflammatory response is brought on by such pain stimulation.[16, 22] In this method by stimulating the chemo-sensitive nociceptors, diluted acetic acid caused the animals to writhe.[23] In the current study of pain-relieving potential analysis by using a writhing model induced by acetic acid, an elevated writhing number was shown by the control group whereas the investigated extracts at doses 200 and 400 mg/kg body weight, reduced writhing significantly. Also, the standard drug Diclofenac sodium (50mg/kg), showed an extremely substantial decrease ($p<0.001$) in abdominal contraction (i.e. % inhibition of writhing) when compared with control. This writhing method confirmed the peripheral analgesic activity of the tested extracts, with n-hexane fraction of *Firmiana colorata* (NHFC) at a higher dose (400 mg/kg) demonstrating the maximum writhing inhibition, indicating significant analgesic potential. The dichloromethane fraction of *Firmiana colorata* (DMFC) showed a moderate effect, while crude methanol leaf extract of *Firmiana colorata*

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(MEFC) had minimal impact. All extracts exhibited a dose-dependent relationship, ranked as NHFC > DMFC > MEFC, suggesting that NHFC is particularly promising for analgesic applications. (Table 2 and Figure 1).

Table 2: Analgesic efficacy of the examined extracts of *F.colorata* leaves' on acetic acid-induced writhing test

Group (Dose mg/kg)	Number of Writhing (Mean ± SEM)	% of Inhibition of Writhing
Control	47.2 ± 1.74	0
Standard	4.6 ± 0.68***	90.25
MEFC 200	26.6 ± 0.93***	43.64
MEFC 400	20.6 ± 0.93***	56.35
NHFC 200	11.6 ± 1.03***	75.42
NHFC 400	8.6 ± 0.81***	81.78
DMFC 200	16 ± 1.84***	66.10
DMFC 400	12 ± 0.71***	74.58

The mean ± SEM (Standard error of Mean) was used to express the results. Statistical significances were expressed as *p<0.05, **p<0.01, ***p<0.001 in relation to the control group. "Statistical Package for Social Science" (SPSS, Version 16.0, IBM Corporation, NY) was used for analyzing the results statistically by using one-way ANOVA and a post hoc Dunnett's test. MEFC= Crude methanol leaf extract of *F.colorata*. NHFC= n-hexane fraction of crude methanol leaf extract of *F. colorata*. DMFC= Dichloromethane fraction of crude methanol leaf extract of *F.colorata*.

Paw-licking assay induced by formalin Two distinct phases were typically involved in the paw-licking assay induced by formalin: a late tonic phase that appeared 15 to 30 minutes post formalin injection and may have been connected to the of inflammatory mediators' release, whereas direct stimulation of nociceptors was represented by an early phase that was seen in the first 5 minutes.[24] In this test, the sample revealed their efficacy to give a more peripheral analgesic effect than central, as the experimental extracts at both doses significantly reduced (***p <0.001) the span of time mice spent licking during the study's late phase than that of study's early phase in comparison to the control group. This test highlighted NHFC's mild central analgesic effects during the early phase and strong peripheral activity in the late phase. In contrast, DMFC displayed limited central effects but moderate peripheral efficacy, and MEFC showed minimal analgesic activity overall. Morphine served as an effective reference, validating the assay's reliability.

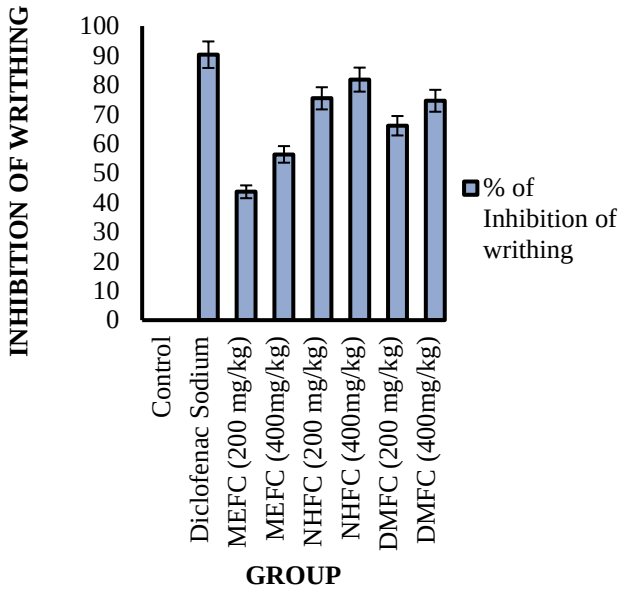

Figure 1: Screening of Analgesic activity of crude methanol extract of *F.colorata* and its solvent fraction by calculating percentage (%) inhibition of writhing by using acetic acid induced writhing method. MEFC= Crude methanol leaf extract of *F.colorata*. NHFC= n-hexane fraction of crude.

These findings suggest that the analgesic properties of NHFC may be explained by the phytochemicals present in the extracts, such as flavonoids and tannins. Future research should focus on isolating these compounds and elucidating their mechanisms of action to enhance understanding and potential therapeutic applications. (Table 3 and Figure 2).

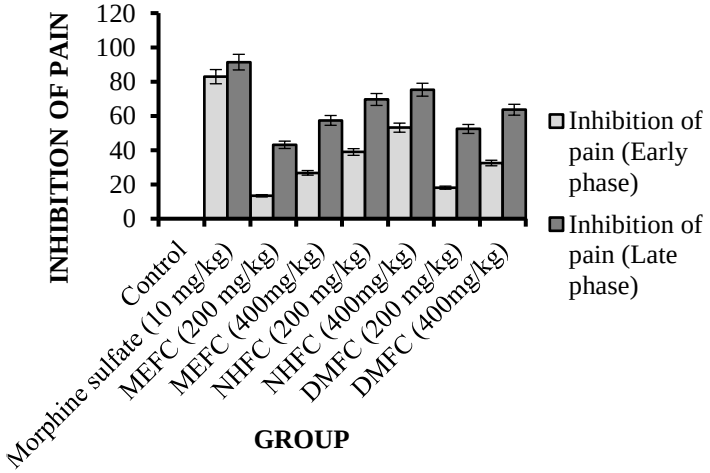

Figure 2: Screening of Analgesic activity of crude methanol extract of *F. colorata* and its solvent fraction by calculating percentage (%) inhibition of pain at different intervals of time using formalin-induced paw licking test. MEFC= Crude methanol leaf extract of *F.colorata*. NHFC= n-hexane fraction of crude methanol leaf extract of *F. colorata*. DMFC= Dichloromethane fraction of crude methanol leaf extract of *F.colorata*.

Table 3: Analgesic efficacy of the examined extracts of *F.colorata* leaves' on formalin-induced paw licking test

Group (Dose mg/kg)	0-5 minutes (Early Phase)		15-30 minute (Late Phase)	
	Paw-licking time (s)	Inhibition of pain (%)	Paw-licking time (s)	Inhibition of pain (%)
Control	50.97 ± 3.82	-	62.44 ± 4.21	-
Standard	8.71 ± 0.61***	82.91	5.39 ± 0.54***	91.37
MEFC 200	44.15 ± 1.96	13.38	35.50 ± 3.33***	43.14
MEFC 400	37.32 ± 3.61*	26.78	26.61± 2.98***	57.38
NHFC 200	31.12 ± 2.25***	38.94	18.99 ± 1.59***	69.59
NHFC 400	23.88 ± 2.99***	53.15	15.42 ± 2.35***	75.30
DMFC 200	41.73 ± 2.61	18.13	29.73 ± 2.88***	52.39
DMFC 400	34.40 ± 3.26**	32.51	22.72 ± 2.51***	63.61

The mean ± SEM (Standard error of Mean) was used to express the results. Statistical significances were expressed as *p<0.05, **p<0.01, ***p<0.001 in relation to control. "Statistical Package for Social Science" (SPSS, Version 16.0, IBM Corporation, NY) was used to analyze the results statistically by one-way ANOVA and a post hoc Dunnett's test. MEFC= Crude methanol leaf extract of *F.colorata*. NHFC= n-hexane fraction of crude methanol leaf extract of F. colorata. DMFC= Dichloromethane fraction of crude methanol leaf extract of *F.colorata*.

Evaluation of antipyretic effect

In the pyrexia model caused by brewer's yeast, an inflammatory response that resulted in fever in mice was due to the proteins found in yeast.[25] In this test, in comparison to the control, the reference standard Morphine sulfate (10 mg/kg) and MEFC and its NHFC and DMFC fractions at both doses (200 and 400 mg/ kg) decreased the rectal temperature of test animals significantly ($p<0.001$) on total (4h) experimental period. This study evaluates the antipyretic effects, demonstrating a substantial dose-dependent reduction in elevated body temperature. In contrast to the control group, which showed increased temperatures, all three investigated extracts highlight their potential as a therapeutic agent. Paracetamol, a standard antipyretic, effectively reduced fever within one hour, serving as a reference point for extract efficacy. The comparable reduction in pyrexia, especially at higher doses of NHFC, suggests it may be an effective alternative to conventional treatments. Additionally, MEFC and DMFC exhibited potential in lowering pyrexia at a higher dose and these outcomes were similar to the conventional medicine paracetamol. Therefore, blocking the synthesis of prostaglandins like paracetamol does, maybe a possible way of having antipyretic action.[26] The presence of flavonoids, saponins, glycosides, and tannins in MEFC likely contributes to its antipyretic effects, as these compounds have been shown to inhibit key inflammatory enzymes.[27] These findings support the hypothesis that the extracts modulate inflammatory pathways to exert antipyretic effects. Overall, this research underscores the promise of *F.colorata* as a natural antipyretic agent, warranting further investigation into its active compounds and mechanisms to develop new therapeutic options for fever management. (Table 4 and Figure 3)

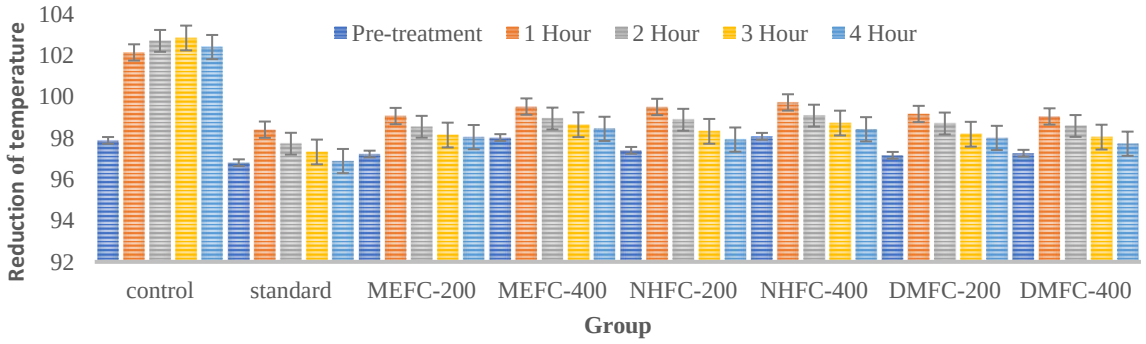

Figure 3: Screening of Antipyretic potential of crude methanol extract of *F.colorata* and its solvent fraction by recording body temperature of mice at different time intervals using Brewer's yeast-induced pyrexia method. MEFC= Crude methanol leaf extract of *F.colorata*. NHFC= n-hexane fraction of crude methanol leaf extract of *F.colorata*. DMFC = Dichloromethane fraction of crude methanol leaf extract of *F.colorata*.

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Table 4: Antipyretic efficacy of the examined extracts of *F.colorata* leaves' on brewer's yeast-induced pyrexia test

Group (Dose mg/kg)	Pre-treatment Temperature(ºF)		Post-treatment Temperature(ºF) (% of reduction of pyrexia)			
	Normal rectal temperature (°F)	After Yeast Administration	1 h	2 h	3 h	4 h
Control	97.88± 0.50	101.4 ± 0.42	102.14±0.37	102.7±0.22	102.84 ±0.18	102.4±0.23
Standard	96.80 ± 0.44	100.76 ± 0.31	98.4±0.39*** (56.59%)	97.72±0.34*** (76.77%)	97.32±0.45*** (86.87%)	96.88±0.41*** (97.98%)
MEFC 200	97.22 ± 0.61	100.26 ± 0.23	99.06±0.23*** (39.47%)	98.54±0.21*** (56.58%)	98.14±0.21*** (69.74%)	98.04±0.37*** (73.03%)
MEFC 400	98.02 ± 0.43	100.66 ± 0.14	99.52 ±0.16*** (43.18%)	98.94±0.10*** (65.15%)	98.64±0.15*** (76.51%)	98.44±0.17*** (84.09%)
NHFC 200	97.40 ± 0.25	101.08 ±0.28	99.50±0.29*** (42.93%)	98.88±0.27*** (59.78%)	98.32±0.17*** (75.00%)	97.92±0.17*** (85.87%)
NHFC 400	98.08 ± 0.31	101.16±0.26	99.72± 0.27*** (46.75%)	99.08±0.29*** (67.53%)	98.72±0.27*** (79.22%)	98.42± 0.26*** (88.96%)
DMFC 200	97.16 ± 0.42	100.4±0.20	99.16± 0.26*** (38.27%)	98.70±0.23*** (52.47%)	98.18±0.26*** (68.52%)	98.0±0.31*** (74.07%)
DMFC 400	97.26 ± 0.38	100.36±0.36	99.04±0.38*** (42.58%)	98.58±0.31*** (57.42%)	98.04±0.33*** (74.84%)	97.72± 0.32*** (85.16%)

The mean ± SEM (Standard error of Mean) was used to express the results. Statistical significances were expressed as *p<0.05, **p<0.01, ***p<0.001 in relation to control. "Statistical Package for Social Science" (SPSS, Version 16.0, IBM Corporation, NY) was used to analyze the results statistically by one-way ANOVA and a post hoc Dunnett's test. MEFC= Crude methanol leaf extract of *F.colorata*. NHFC= n-hexane fraction of crude methanol leaf extract of *F. colorata.* DMFC= Dichloromethane fraction of crude methanol leaf extract of *F.colorata*.

In vitro antioxidant studies of Firmiana colorata extracts

Antioxidants, also referred to as free radical scavengers, are substances that combine and neutralize free radicals to prevent them from harming bodily cells.[28] From the literature survey, it was found that the *F. colorata* leaf extract in methanol was assessed for its activity against oxidative stress and was proved to be a significant antioxidant.[7] Current study assessed the ability of different extracts to fend off oxidative stress using the DPPH free radical and H_2O_2 scavenging assays.

Assay for DPPH radical scavenging: DPPH has the capability of transforming into a stable diamagnetic molecule by accepting an electron or hydrogen atom and is considered a stable free radical. This property is utilized to evaluate the antioxidant potential of the plant extract.[29] Analysis of the antioxidant potential of the investigated extracts was carried out using DPPH assay. The results indicated that the extracts with the higher percentages (%) of scavenging effect and a lower IC_{50} value have a higher potential to protect against oxidative stress. In this assay, the reference standard ascorbic acid (AA) showed a scavenging effect of 91.77 % at the highest concentration. The IC_{50} value of AA was much less (0.093µg/ml) which showed its highly effective potential as a free radical scavenger. DMFC exhibited the maximum capacity to scavenge free radicals with a low IC_{50} value, suggesting potent antioxidant properties. In contrast, the control showed no activity, underscoring the importance of active extracts. MEFC outperformed NHFC, indicating differing antioxidant capacities. (Table 5 and Figure 4). The observed antioxidant potential of the extracts may be linked to flavonoids, as flavonoids are known for their ability to neutralize free radicals and prevent oxidative stress.[30]

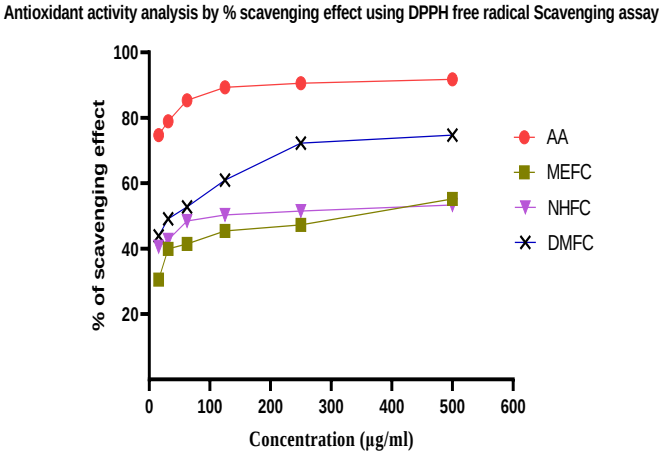

Figure 4: Determination of the antioxidant activity of different concentrations of crude methanol extract of *Firmiana colorata* and its solvent fraction by comparing IC_{50} value using DPPH free radical scavenging assay. AA=Ascorbic acid, MEFC= Crude methanol leaf extract of *F.colorata*. NHFC= n-hexane fraction of crude methanol leaf extract of *F.colorata*. DMFC= Dichloromethane fraction of crude methanol leaf extract of *F.colorata*.

Table 5: Antioxidant efficacy of the examined extracts of *F.colorata* leaves' on DPPH free radical scavenging assay

Group	Equation	R^2	IC_{50} (µg/ml)
Control	-	-	-
Standard (AA)	y = 11.783x + 62.175	0.9241	0.093
MEFC	y = 14.179x + 15.696	0.9372	262.63
NHFC	y = 8.7657x + 30.75	0.9364	157.06
DMFC	y = 21.995x +16.129	0.9692	34.67

AA=Ascorbic acid, MEFC= Crude methanol leaf extract of *F.colorata*. NHFC= n-hexane fraction of crude methanol leaf extract of *F. colorata*. DMFC= Dichloromethane fraction of crude methanol leaf extract of *F.colorata*.

H_2O_2 Scavenging Assay: The imbalance between pro-oxidants and antioxidants plays a crucial part in the development of many diseases. Numerous studies have shown that plants' medicinal properties are primarily linked to their phytochemicals which have antioxidant properties.[31] One important oxygen metabolite generated by oxidase enzymes and active phagocytes is hydrogen peroxide H_2O_2.[32] Its antioxidant potency can be measured with a straightforward, precise, and sensitive technique. In this study, the reference standard was ascorbic acid which showed the highest H_2O_2 scavenging potential of 89.91% at the highest concentration investigated. It showed good H_2O_2 scavenging effectiveness even at the lowest concentration investigated, whereas control distilled water did not show any H_2O_2 scavenging effect. Among the investigated extracts MEFC demonstrated higher H_2O_2 scavenging capacity, whereas DMFC showed moderate scavenging effect. NHFC had the least scavenging ability, suggesting limited antioxidant potential, while AA remained a strong scavenger in both assays. These findings highlight the diverse mechanisms of antioxidant activity in natural extracts and warrant further exploration of their active compounds. (Table 6 and Figure 5).

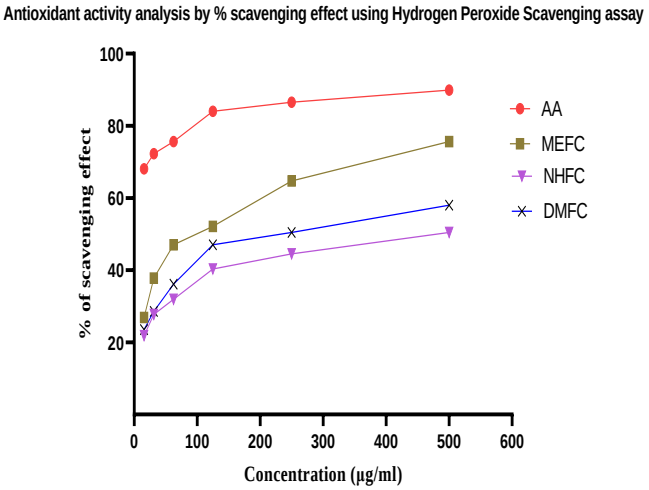

Figure 5: Determination of the antioxidant activity of different concentrations of crude methanol extract of *F. colorata* and its solvent fraction by comparing IC_{50} value using H_2O_2 scavenging assay. AA=Ascorbic acid, MEFC= Crude methanol leaf extract of *F.colorata*. NHFC= n-hexane fraction of crude methanol leaf extract of *F.colorata*. DMFC= Dichloromethane fraction of crude methanol leaf extract of *F.colorata*.

Table 6: Antioxidant efficacy of the examined extracts of *F.colorata* leaves' on hydrogen peroxide scavenging assay

Group	Equation	R^2	IC_{50} (µg/ml)
Control	-	-	-
Standard (AA)	y = 15.229x + 49.769	0.9769	01.04
MEFC	y = 31.27x -10.164	0.99	83.95
NHFC	y = 19.141x -1.1236	0.9938	468.70
DMFC	y = 23.609x -5.3383	0.9861	220.77

AA=Ascorbic acid, MEFC= Crude methanol leaf extract of *F.colorata*. NHFC= n-hexane fraction of crude methanol leaf extract of *F.colorata*. DMFC= Dichloromethane fraction of crude methanol leaf extract of *F.colorata*.

Conclusion

This study elucidates the pharmacological potential of *F. colorata* methanol leaf extracts, demonstrating significant analgesic, antipyretic, and antioxidant properties. The pronounced analgesic activity, particularly of the NHFC extract, underscores its promise as an alternative or complementary option for pain management, validated through both peripheral and mild central effects. The antipyretic evaluation reveals that *F. colorata* effectively reduces fever in a dose-dependent manner, suggesting its viability as a natural treatment for hyperpyrexia, especially in contexts where conventional antipyretics may be contraindicated. Furthermore, the antioxidant potential of the extracts, highlighted through their strong free radical scavenging abilities, positions *F. colorata* as a valuable candidate for addressing oxidative stress-related disorders. The diverse mechanisms underlying these effects warrant further exploration of the specific bioactive compounds present in the plant. Overall, this research not only reinforces the therapeutic potential of *F. colorata* in traditional medicine but also paves the way for future studies aimed at isolating its active constituents. Such efforts might result in the creation of novel natural therapeutics, bridging gaps between traditional practices and modern pharmacology, and ultimately enhancing our understanding of plant-based interventions in health care.

Conflict of Interest

Authors declare no conflict of interest.

Authors' Declaration

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

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