



Isolation, Partial Purification and Median Lethal Dose of Antipyretic Agent from *Khaya senegalensis* Leaf Extracts

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Abstract

This research was designed to evaluate the antipyretic activity of *K. senegalensis* leaf extract with the view of isolating and identifying the active components. The *K. senegalensis* leaf was extracted using 90% methanol and further fractionated with hexane, ethylacetate, n-butanol and distilled water. The qualitative phytochemical screening was carried out using standard methods. The crude extract and the fractions were screened for antipyretic activity using 15%w/v brewer's yeast induced pyrexia on Albino rats. The components of the most active fraction were further separated using column and thin layer chromatographic techniques on silica gel. LD₅₀ of the most active fraction was determined using probit analysis. The results of the phytochemical screening revealed the presence of tannins, phenols, steroids and cardiac glycosides in both the crude extract and its fractions. The crude extract at 400 mg/kg b.w. showed the highest antipyretic activity compared to the other doses tested. Hexane fraction showed the highest antipyretic activity among the other fractionated extracts. The LD₅₀ of the hexane fraction was found to be 831.76 mg/kg b.w. The column chromatographic separation of the hexane extract yielded 60 fractions (F1 to F60). After TLC separation, fractions with similar profile were pooled together yielding eleven (11) pooled fractions (PF1 to PF11). Antipyretic activities of the pooled fractions showed that PF8 exhibited the highest activity. These findings suggested that, *K. senegalensis* leaf has significant antipyretic activity which can be considered for the development of antipyretic agent from natural resources.

Introduction

Pyrexia of any type is the most common reasons of physicians' consultation in various health care delivery system. Most pathological conditions are associated with pyrexia, pains and inflammation. The widespread and growing use of traditional medicine (example *Khaya senegalensis*) of unproven safety and efficacy has created public health challenges in terms of policy, safety, efficacy, quality and rational use (WHO, 2002). Additionally, the development of antipyretic agents that are more effective, safer and readily affordable is a challenging tasks for researchers.

Medicinal plants are used world-wide to treat many diseases and are valuable sources of compounds for synthesis of modern drugs (Eto, 2012). Many rural dwellers depend largely on herbs for the treatment of painful conditions, inflammation and fever. However, the unknown side effect of these herbs can pose health problems (Omogbai et al., 2010; Shekthawat and Vijayvergia, 2010).

Synthetic drugs like paracetamol, ibuprofen, aspirin, etc., have been developed to manage pyrexia, yet they still have some limitations such as its toxicity. Therefore, this prompts a search for safe, accessible and affordable antipyretic medication from natural resources

Methods

Experimental Animals

During the month of March, 2019, healthy albino rats weighing between 120g and 180g, of both sexes, were obtained from the Usmanu Danfodiyo University Animal House in Sokoto. In well-ventilated cages, the animals were transferred to Aliero, where they were housed in the Animal House of the Faculty of Life Sciences at Kebbi State University of Science and Technology. After acclimatizing for fourteen (14) days prior to the start of the experiment, the rats were kept in clean cages for the duration of the study. All of the rats were given full access to food and water, which was a normal rat chow.

Collection and Identification of the *Khaya senegalensis* Leaf

The fresh leaf of *K. senegalensis* was harvested on April 24th, 2018 in Wamakko, Sokoto State, Nigeria, and has been stored there since. An expert in plant science and biotechnology from the Department of Plant Science and Biotechnology at Kebbi State University of Science and Technology (Kebbi, Nigeria), where a voucher specimen (61A) had been placed, then authenticated the specimen.

Plant Preparation and Extraction

In order to properly dry them, they were cleaned with tap water before being allowed to air dry at room temperature under cover in the shade. We ground it up using a mortar and pestle until it was the consistency of coarse powder. Over a 48-hour period, 500 grams (500g) of the material was macerated in 2500ml of 90 percent methanol. An electric oven set at 45 degrees Celsius was used to evaporate the filtrate after it had been filtered through a muslin cloth and dried. The dried extract was stored in clean, airtight containers and maintained at 80 degrees Celsius in a refrigerator until it was needed (Dupont et al., 2006).

Fractionation of the Crude Methanol Extract

After fractionating the crude Methanol extract of the *K. senegalensis* leaf with organic solvents in increasing order of polarity, the crude Methanol extract was re-extracted using the same solvents in the same order. A total of two hundred grams (200g) of the methanol plant extract was reconstituted in 400 milliliters (400mL) of distilled water in a 1000 milliliter separator funnel, and the mixture was partitioned sequentially with equal volumes of hexane (saturated with water), ethyl-acetate (saturated with water), and n-butanol (saturated with water) to yield the hexane, e Afterwards, each fraction was concentrated to dryness, and the resultant extract was stored refrigerated in an airtight container in preparation for future analysis. Each fraction was reconstituted in distilled water prior to use, and the dry weight (mg/ml) of each fraction was determined. The results were reported as mg/ml.

Qualitative Phytochemical Screening of *Khaya senegalensis* Leaf Extract

Test for Alkaloids

It took 20 minutes on a steam bath to dissolve the extract (0.5g) in 5ml of 10 percent aqueous hydrochloric acid before cooling and filtration. Two milliliters (2ml) of the acidic medium were treated with a few drops of Mayer's reagent to get the desired result (Potassium Mercuric Iodide). The presence of alkaloids was revealed by the formation of a yellow coloured precipitate (Trease & Evans, 1989).

Test for Anthraquinones

In a water bath, the extract (0.5g) was cooked for a few minutes with 10 percent HCl to remove the tannins. It was filtered and let to cool before being used. The filtrate was then treated with an equal amount of chloroform. A few drops of ammonia (10 percent concentration) were added to the mixture and then heated. The presence of anthraquinones was revealed by the formation of a rose-pink color (Sofowora, 1993).

Test for Cardiac Glycosides

It was decided to use the Keller-Kiliani test to identify the presence of cardiac glycosides. In order to treat the crude powder (0.5g), 1ml of a combination containing 5 percent FeCl₃ and glacial acetic acid (99:1 v/v) was added to the mixture. A few drops of concentrated H₂SO₄ were added to this solution to make it more concentrated. The presence of cardiac glycosides was revealed by the appearance of a greenish blue color after a few minutes (Trease & Evans, 1989).

Test for Flavonoids

The extract (0.5g) was dissolved in diluted NaOH before being added to the solution with HCl. The presence of flavonoids was indicated by a yellow solution that became colorless after a period of time (Harborne, 1973).

Test for Glycosides

Extract (0.5g) was hydrolyzed with HCl and neutralized with a solution of sodium hydroxide. A few drops of Fehling solution A and B were added to complete the mixture. The presence of glycosides was suggested by the appearance of a crimson precipitate (Trease & Evans, 1989).

Test for Phenols

It was necessary to boil the extract (0.5g) with 10ml of sulphuric acid and filter it while it was still hot, after which the filtrate was shaken with 5ml of chloroform. It was necessary to pipette the chloroform layer into another test tube and then add 1ml of weak ammonia to it. The color of the resultant solution was examined to see whether it changed (Sofowora, 1993).

Test for Saponins

The extract (0.5g) was mixed with 5ml of distilled water before being brought to a boil in a saucepan. It was discovered that saponins were present due to foaming (the appearance of a creamy mist of tiny bubbles) (Sofowora, 1993).

Test for Tannins

It was necessary to combine exactly 0.5g of the extract with water before heating it on a water bath and filtering it. The ferric chloride solution was added to the filtrate in three (3) drops at a time. The presence of tannins was suggested by the presence of a dark green solution (Trease & Evans, 1989).

Test for Terpenoids

The crude extract was diluted in 2ml of chloroform and then evaporated to dryness to remove any residual moisture. In the next step, 2 mL of concentrated H₂SO₄ was added and heated for about 2 minutes. The presence of terpenoids was suggested by the presence of a greyish color (Harborne, 1973).

Antipyretic Study of *K. senegalensis* Leaf Extract

It was determined if the crude methanol extract of *Kigelia senegalensis* leaf and its fractions had antipyretic activity in rats when they were subjected to brewer's yeast-induced pyrexia. The method outlined by Al-Ghamdi (2001) was followed for the purpose of this investigation. The body temperature of each albino rat was measured at 30 minute intervals for 4 hours, with the rectal temperature (RT) being taken every 30 minutes. A 150mg/kg suspension (15 percent w/v) of brewer's yeast (*Saccharomyces cerevisiae*) was injected subcutaneously into the rats at a dose of 1ml/kg body weight per rat, resulting in the induction of fever. After 18 hours after yeast delivery, the rectal temperature of each individual was measured once again. Rats that did not demonstrate a minimum rise in temperature of 0.5°C within 18 hours after yeast injection were excluded from the study. All of the medications were administered orally. Rectal temperatures of all the rats were taken by inserting a digital thermometer into the rectum of each rat and recording the results. Pyretic albino rats of either sex were split into seven (7) groups of four (4) rats each for the crude extract, with the groups being divided as follows:

Group 1 (Normal Control): rats in this group were given normal saline (5ml/kg body weight) orally.

Group 2: rats in this group were (subcutaneously) administered with yeast (1ml/kg body weight of 15% yeast solution) only.

Group 3: rats in this group were (subcutaneously) administered with yeast (1ml/kg body weight of 15% yeast solution) and paracetamol (150mg/kg) orally.

Group 4: rats in this group were (subcutaneously) administered with yeast (1ml/kg body weight of 15% yeast solution) and 100mg/kg body weight the crude extract orally.

Group 5: rats in this group were (subcutaneously) administered with yeast (1ml/kg body weight of 15% yeast solution) and 200mg/kg body weight of the crude extract orally.

Group 6: rats in this group were (subcutaneously) administered with yeast (1ml/kg body weight of 15% yeast solution) and 400mg/kg of the crude extract orally.

Group 7: rats in the group were (subcutaneously) administered with 400mg/kg body weight crude extract only orally.

Antipyretic Study of *K. senegalensis* Leaf Fractions:

Antipyretic test was performed using the fractions and animals were grouped as follows.

Group 1 (Normal Control): rats in this group were (subcutaneously) administered with normal saline (5ml/kg body weight) orally.

Group 2: rats in this group were (subcutaneously) administered with yeast (1ml/kg body weight of 15% yeast solution) only.

Group 3: rats in this group were (subcutaneously) administered with yeast (1ml/kg body weight of 15% yeast solution) and paracetamol (150mg/kg) orally.

Group 4: rats in this group were (subcutaneously) administered with yeast (1ml/kg body weight of 15% yeast solution) and 200mg/kg body weight of Hexane fraction orally.

Group 5: rats in this group were (subcutaneously) administered with yeast (1ml/kg body weight of 15% yeast solution) and 200mg/kg body weight of Ethyl acetate fraction orally.

Group 6: rats in this group were (subcutaneously) administered with yeast (1ml/kg body weight of 15% yeast solution) and 200mg/kg body weight of n-butanol fraction orally.

Group 7: rats in the group were (subcutaneously) administered with 200mg/kg body weight of aqueous fraction orally.

Determination of LD₅₀ of the Most Active Fraction (MAF)

The LD₅₀ of the most active fraction (MAF) was determined using Probit analysis. Fifteen (15) albino rats were randomly divided into five (5) groups of three (3) rats each as follows:

Group 1: received orally 1g/kg body weight of the MAF.

Group 2: received orally 2g/kg body weight of the MAF.

Group 3: received orally 3g/kg body weight of the MAF.

Group 4: received orally 4g/kg body weight of the MAF.

Group 5: received orally 5g/kg body weight of the MAF.

After Administration, each rat was observed for mortality within the first 8hours, 24hours, 48hours and then daily for a period of 14days.

Isolation and Identification of the Active Component(s)

Chromatographic Separation

Techniques such as column and thin layer chromatography on silica gel were used to isolate the components of the most active fraction. An addition of the dried fraction to silica gel (60-120 mesh) in a chromatographic column resulted in the following results: (85cm x 4cm). It was packed with n-hexane and eluted using a mixture of hexane and chloroform (98:02, 95:05, 90:10, 80:20, 70:30, 60:40, 50:50, 1 L each) to get the desired result. They were then dried to dryness and evaluated for homogeneity using TLC and the antipyretic effect, among other methods (in a similar way as describe above). A precoated TLC plate was utilized for the thin-layer chromatography (TLC) analysis. 30 minutes at 105 degrees Celsius was sufficient to activate the plates In order to identify the chemicals, spots were created and tested.

Antipyretic Activities of the Chromatographic Fractions

Antipyretic test was performed using the chromatography pooled fractions based on TLC. The animals were grouped as follows.

Group 1 (Normal Control): rats in this group were given normal saline (5ml/kg body weight) orally.

Group 2: rats in this group were (subcutaneously) administered with yeast (1ml/kg body weight of 15% yeast solution).

Group 3: rats in this group were (subcutaneously) administered with yeast (1ml/kg body weight of 15% yeast solution) and paracetamol (150mg/kg body weight) orally.

Group 4: rats in this group were (subcutaneously) administered with yeast (1ml/kg body weight of 15% yeast solution) and chromatographic fraction 1 (100mg/kg body weight) orally.

Group 5: rats in this group were (subcutaneously) administered with yeast (1ml/kg body weight of 15% yeast solution) and chromatographic fraction 2 (100mg/kg body weight) orally.

Group 6: rats in this group were (subcutaneously) administered with yeast (1ml/kg body weight of 15% yeast solution) and chromatographic fraction 3 (100mg/kg body weight) orally.

Group 7: rats in this group were (subcutaneously) administered with yeast (1ml/kg body weight of 15% yeast solution) and chromatographic fraction 4 (100mg/kg body weight) orally.

Group 8: rats in this group were (subcutaneously) administered with yeast (1ml/kg body weight of 15% yeast solution) and chromatographic fraction 5 (100mg/kg body weight) orally.

Group 9: rats in this group were (subcutaneously) administered with yeast (1ml/kg body weight of 15% yeast solution) and chromatographic fraction 6 (100mg/kg body weight) orally.

Group 10: rats in this group were (subcutaneously) administered with yeast (1ml/kg body weight of 15% yeast solution) and chromatographic fraction 7 (100mg/kg body weight) orally.

Group 11: rats in this group were (subcutaneously) administered with yeast (1ml/kg body weight of 15% yeast solution) and chromatographic fraction 8 (100mg/kg body weight) orally.

Group 12: rats in this group were (subcutaneously) administered with yeast (1ml/kg body weight of 15% yeast solution) and chromatographic fraction 9 (100mg/kg body weight) orally.

Group 13: rats in this group were (subcutaneously) administered with yeast (1ml/kg body weight of 15% yeast solution) and chromatographic fraction 10 (100mg/kg body weight) orally.

Group 14: rats in this group were (subcutaneously) administered with yeast (1ml/kg body weight of 15% yeast solution) and chromatographic fraction 11 (100mg/kg body weight) orally.

Identification of the Active Compound

The active compound was identified using GC-MS, FTIR and UV analytical techniques.

Data Analysis

The results were expressed as mean \pm standard error of the mean (S.E.M). The data obtained was analysed statistically using one-way analysis of variance (ANOVA) and significant difference was tested at $P < 0.05$ using SPSS (version 20) statistical software

Results and Discussion

Qualitative Phytochemical Composition

The presence of flavonoids, phenols, tannins, saponins, alkaloids, terpenoids, steroids, and cardiac glycosides was discovered in the crude methanol leaf extract of *K. senegalensis* after a qualitative phytochemical screening was performed (Table 1). The presence of phlobatannins and anthraquinones in the crude extract was not determined. It was discovered that the n-hexane fraction included phenols, tannins, alkaloids, terpenoids, steroids, and cardiac glycosides, among other things. While this was happening, the ethylacetate fraction obtained from the leaves of *K. senegalensis* revealed the presence of phenols, tannins, terpenoids, steroid and cardiac glycosides, as well as other compounds. The screening of the n-butanol fraction and the aqueous fractions of the *K. senegalensis* leaf showed the presence of phenols, tannins, saponins, steroids, and cardiac glycosides in both the n-butanol fraction and the aqueous fraction. Additionally, the n-butanol fraction included flavonoids in addition to the aforementioned components.

Table 1. Qualitative Phytochemical Composition of *Khaya senegalensis* Leaf

| Phytochemical | Crude Methanol Extract | n-Hexane Fraction | Ethylacetate Fraction | n-Butanol Fraction | Aqueous Fraction |
|---------------|------------------------|-------------------|-----------------------|--------------------|------------------|
| Flavonoids | + | - | - | + | - |
| Phenols | + | + | + | + | + |
| Tannins | + | + | + | + | + |
| Saponins | + | - | - | + | + |
| Phlobatannins | - | - | - | - | - |

| | | | | | |
|--------------------|---|---|---|---|---|
| Alkaloids | + | + | - | - | - |
| Terpenoids | + | + | + | - | - |
| Steroids | + | + | + | + | + |
| Anthraquinones | - | - | - | - | - |
| Cardiac Glycosides | + | + | + | + | + |

Note: + = Present, - = Not detected

Antipyretic Effect of the Crude Methanol Extract

As shown in Table 2, the antipyretic effect of *K. senegalensis* leaves extract dissolved in methanol on albino rats has been studied. When comparing the treatment groups to the control group at 0 hours, there was no statistically significant difference in rectal temperature between the treated groups and the control group ($P>0.05$). When compared to the usual control group, which got just 5mg/kg distilled water, subcutaneous injection of brewer's yeast substantially raised ($P0.05$) the rectal temperature in all of the groups that received it after 18 hours. When compared to the control group that did not receive any treatment, the rectal temperature of albino rats in the extract and drug treated groups was substantially lower ($P0.05$) two (2) hours after treatment. Similarly, a similar pattern was seen at the third (3) hour and the fourth (4) hour following treatment, according to the findings.

Antipyretic Effect of the Fractionated Extracts

Using albino rats, we investigated the antipyretic effect of the fractions. The results revealed that the rectal temperature of the rats was substantially lower ($P0.05$) in the normal control group when compared to the other groups at 0 hours. When compared to the usual control group, which got just 5mg/kg distilled water, subcutaneous injection of brewer's yeast substantially raised ($P0.05$) the rectal temperature in all of the groups that received it after 18 hours.

The temperature of the positive control group (the group that received just yeast treatment) was substantially higher ($P0.05$) than the temperature of the other groups. When the temperature of the paracetamol-treated group was compared to that of the normal control group, the difference was statistically significant ($P0.05$). This pattern persisted for 2 and 3 hours after the start of therapy, respectively. At four (4) hours following treatment, the normal control and paracetamol treated groups had substantially lower ($P0.05$) levels of nitric oxide than the extract treated groups. The antipyretic activity of the hexane fraction was found to be the greatest in the group treated with it, while the aqueous fraction was found to be the lowest (Table 3).

Table 2. Antipyretic Effect of Crude Methanol Leaves Extract of *K. senegalensis* on Albino Rats

| Group Treatment | Before Treatment | | After Treatment | | | |
|------------------------------------|-------------------------|-------------------------|-------------------------|--------------------------|--------------------------|--------------------------|
| | Temperature 0Hrs (°C) | Temperature 18Hrs (°C) | Temperature 1Hr (°C) | Temperature 2Hrs (°C) | Temperature 3Hrs (°C) | Temperature 4Hrs (°C) |
| Normal Saline (0.9%) | 36.35±0.18 ^a | 36.20±0.11 ^a | 36.38±0.09 ^a | 36.25±0.13 ^{ab} | 36.35±0.19 ^{ab} | 36.20±0.09 ^{ab} |
| Yeast only (10mg/kg) | 36.73±0.14 ^a | 37.10±0.18 ^b | 37.10±0.39 ^a | 37.58±0.17 ^c | 37.93±0.13 ^c | 38.03±0.08 ^c |
| Yeast and Crude Extract (100mg/kg) | 36.93±0.21 ^a | 37.23±0.21 ^b | 36.45±0.16 ^a | 36.18±0.09 ^{ab} | 36.08±0.11 ^{ab} | 35.95±0.06 ^{ab} |

| | | | | | | |
|------------------------------------|-------------------------|-------------------------|-------------------------|--------------------------|--------------------------|--------------------------|
| Yeast and Crude Extract (200mg/kg) | 36.88±0.43 ^a | 37.23±0.21 ^b | 37.03±0.40 ^a | 36.78±0.42 ^b | 36.55±0.43 ^b | 36.45±0.49 ^b |
| Yeast and Crude Extract (400mg/kg) | 36.65±0.10 ^a | 37.65±0.15 ^b | 36.73±0.27 ^a | 36.35±0.25 ^{ab} | 36.20±0.23 ^{ab} | 35.93±0.16 ^{ab} |
| Yeast and Paracetamol (150mg/kg) | 36.63±0.21 ^a | 37.43±0.24 ^b | 36.20±0.24 ^a | 35.93±0.18 ^a | 35.68±0.17 ^a | 35.53±0.17 ^a |

Values are expressed as mean ± standard error of mean. Mean values having common superscript letters in a column are not significantly different (p<0.05) (using ANOVA followed by Duncan’s multiple range test)

Table 3. Antipyretic Effect of *K senegalensis* leaf Fractionated Extracts on Albino Rats

| Group Treatment | Before Treatment | | After Treatment | | | |
|---|---------------------------|---------------------------|---------------------------|--------------------------|---------------------------|---------------------------|
| | Temperature 0Hr (°C) | Temperature 18Hrs (°C) | Temperature 1Hr (°C) | Temperature 2Hrs (°C) | Temperature 3Hrs (°C) | Temperature 4Hrs (°C) |
| Normal Saline (0.9%) | 36.35±0.17 ^a | 36.20±0.10 ^a | 36.38±0.09 ^{ab} | 36.25±0.13 ^{ab} | 36.35±0.18 ^{ab} | 36.20±0.09 ^{ab} |
| Yeast only (10mg/kg) | 36.73±0.14 ^{abc} | 37.10±0.18 ^b | 37.10±0.39 ^{bcd} | 37.57±0.17 ^{cd} | 37.93±0.13 ^e | 38.03±0.06 ^e |
| Yeast and Hexane Fraction (200mg/kg) | 36.38±0.29 ^a | 37.28±0.21 ^b | 36.93±0.17 ^{abc} | 36.78±0.13 ^{bc} | 36.45±0.14 ^{abc} | 36.25±0.13 ^{abc} |
| Yeast and Ethyl acetate Fraction (200mg/kg) | 37.00±0.18 ^{bc} | 38.38±0.46 ^d | 37.85±0.36 ^d | 37.73±0.41 ^d | 37.23±0.31 ^{cde} | 36.98±0.30 ^{cd} |
| Yeast and Butanol Fraction (200mg/kg) | 37.28±0.19 ^c | 38.20±0.13 ^{cd} | 37.68±0.22 ^{cd} | 37.30±0.29 ^{cd} | 36.98±0.31 ^{bcd} | 36.40±0.20 ^{bc} |
| Yeast and Aqueous Fraction (200mg/kg) | 36.60±0.09 ^{ab} | 37.88±0.29 ^{bcd} | 37.85±0.34 ^d | 37.80±0.36 ^d | 37.65±0.43 ^{de} | 37.45±0.45 ^{de} |
| Yeast and Paracetamol (150mg/kg) | 36.63±0.21 ^{ab} | 37.43±0.24 ^{bc} | 36.20±0.23 ^a | 35.91±0.17 ^a | 35.68±0.17 ^a | 35.53±0.17 ^a |

Values are expressed as mean ± standard error of mean. Mean values having common superscript letters in a column are not significantly different (p<0.05) (using NOVA followed by Duncan’s multiple range test)

TLC Profile of the Chromatographic Fractions of the Hexane Extract

A total of 60 chromatographic fractions (CCF) were eluted at the end of the column chromatography. Based on the TLC profile of the 60 chromatographic fractions, a total of eleven (11) pooled fractions (PF1 - PF11) were obtained (Plate 1).

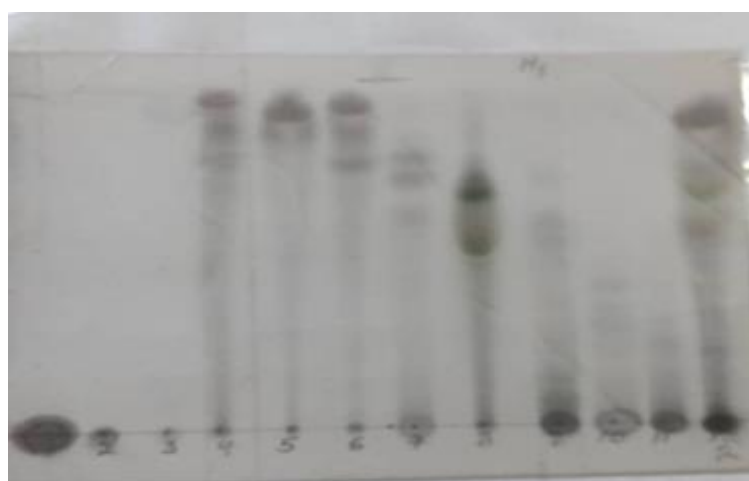


Figure 1. Chromatogram of Pooled Fractions

Antipyretic Effect of the Chromatographic Pooled Fractions

Table 5 shows the antipyretic activity of chromatographic pooled fractions of the hexane extract of *K. senegalensis* on albino mice. The extract of *K. senegalensis* was extracted using a hexane extraction method. Compared to the other groups of albino rats, the rectal temperature of those given with Fraction 10 was significantly higher (P0.05) at 0 hours. When compared to the usual control group, which got just 5mg/kg distilled water, subcutaneous injection of brewer's yeast substantially raised (P0.05) the rectal temperature in all of the groups that received it after 18 hours.

A substantial difference (P0.05) existed between the rectal temperatures of the different groups one (1) hour after treatment with their respective doses. The temperature of the positive control was considerably higher than (P0.05) that of the other groups. Just the group that received only yeast showed a statistically significant increase (P0.05) compared to the other groups two (2) hours after treatment. This trend continued three (3) hours after treatment, and four (4) hours after treatment, there was no statistically significant difference (P>0.05) between the normal control and the pooled fraction 8, indicating that the fraction 8 has the highest antipyretic activity of the three fractions tested.

Median Lethal Dose (LD50) of the Hexane Fraction

This is presented in Table 4. The corresponding Log Dose at 50% probit of mortality (Figure 1) is 2.92; hence the LD50 dose of the hexane fraction was calculated as follows: Antilog of 2.92 = 102.92 = 831.76mg/kg.

Table 4. Probit Analysis for Determination of LD50 of Hexane Fraction

| Group | Dose (mg/kg b.w.) | Average weight (g) | Average dose administered (mg) | Log dose | Dead Total | % Death | % Corrected* | Probit value |
|-------|-------------------|--------------------|--------------------------------|----------|------------|---------|--------------|--------------|
| 1 | 1000 | 164.9 | 164.9 | 2.23 | 0/3 | 0 | 8.33 | 3.59 |
| 2 | 2000 | 188.0 | 376.0 | 2.59 | 0/3 | 0 | 8.33 | 3.59 |
| 3 | 3000 | 177.6 | 532.8 | 2.73 | 0/3 | 0 | 8.33 | 3.59 |
| 4 | 4000 | 208.7 | 834.8 | 2.92 | 1/3 | 33.3 | 33.3 | 4.56 |
| 5 | 5000 | 234.1 | 1,170.5 | 3.07 | 3/3 | 100 | 91.67 | 6.41 |

*Corrected formulae: 0% death = $100 \left(\frac{0.25}{n} \right) = 100 \left(\frac{0.25}{3} \right) = 8.33$ (Premendranet *al.*, 2011).

100% dead = $100 \left(\frac{n-0.25}{n} \right) = 100 \left(\frac{3-0.25}{3} \right) = 91.66$ Where n is the number of rats in a group.

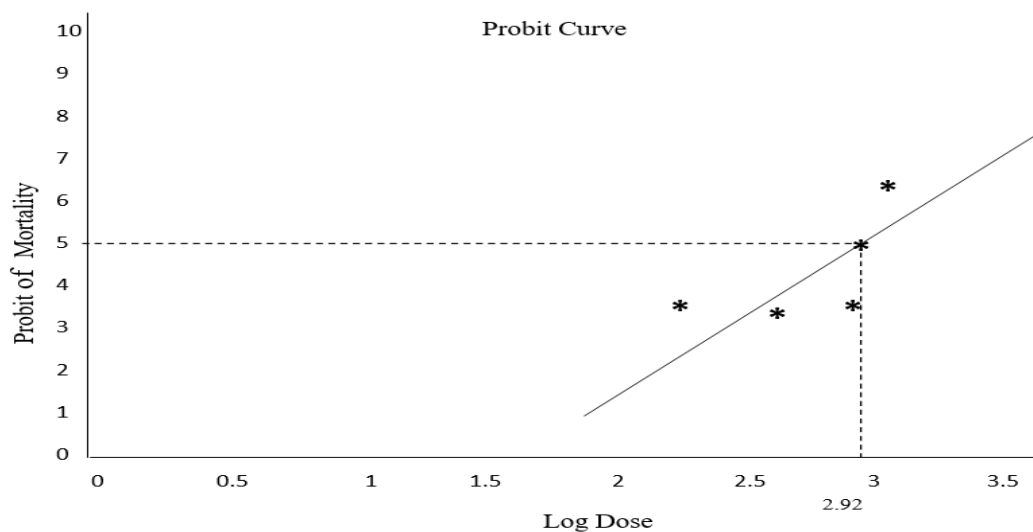


Figure 2. Probit Curve for Determination of LD50 of Hexane Fraction

Table 5. Antipyretic Effect of Pooled Chromatographic Fractions of *K. Senegalensis* leaf extract on Albino Rats

| Group Treatment | Before Treatment | | After Treatment | | | |
|----------------------------------|----------------------------|---------------------------|--------------------------|---------------------------|---------------------------|----------------------------|
| | Temperature 0Hrs(°C) | Temperature 18Hrs(°C) | Temperature 1Hrs(°C) | Temperature 2Hrs(°C) | Temperature 3Hrs(°C) | Temperature 4Hrs(°C) |
| Normal Saline | 36.23±0.19 ^{abcd} | 36.10±0.06 ^a | 36.30±0.06 ^a | 36.23±0.19 ^{abc} | 36.47±0.20 ^{ab} | 36.23±0.12 ^{ab} |
| Yeast only (10mg/kg) | 36.67±0.18 ^{abcd} | 37.00±0.21 ^b | 37.43±0.30 ^b | 37.73±0.07 ^d | 37.83±0.12 ^e | 37.97±0.07 ^e |
| Yeast and Paracetamol (150mg/kg) | 36.57±0.29 ^{abcd} | 37.37±0.33 ^{bcd} | 36.30±0.29 ^a | 36.00±0.23 ^{bc} | 35.77±0.20 ^{abc} | 35.57±0.23 ^{abc} |
| Yeast and Fraction 1 | 36.74±0.04 ^{cd} | 37.97±0.06 ^{cde} | 37.89±0.02 ^{bc} | 37.74±0.10 ^e | 37.64±0.10 ^{cd} | 37.10±0.04 ^{def} |
| Yeast and Fraction 2 | 36.39±0.25 ^{abcd} | 37.98±0.03 ^{cde} | 37.96±0.01 ^{bc} | 37.93±0.06 ^e | 37.96±0.03 ^e | 37.94±0.04 ^f |
| Yeast and Fraction 3 | 36.65±0.20 ^{abcd} | 38.04±0.04 ^{de} | 38.19±0.37 ^c | 37.87±0.14 ^e | 37.74±0.13 ^e | 37.15±0.14 ^{def} |
| Yeast and Fraction 4 | 36.04±0.14 ^{ab} | 37.94±0.05 ^{cde} | 37.82±0.11 ^{bc} | 37.85±0.08 ^e | 37.83±0.08 ^e | 37.87±0.03 ^e |
| Yeast and Fraction 5 | 36.71±0.34 ^{bcd} | 37.92±0.05 ^{cde} | 37.74±0.05 ^{bc} | 37.44±0.06 ^{de} | 37.04±0.09 ^{bc} | 36.59±0.05 ^{bcde} |
| Yeast and Fraction 6 | 36.51±0.06 ^{abcd} | 38.11±0.06 ^e | 37.94±0.05 ^{bc} | 37.88±0.02 ^e | 37.88±0.03 ^e | 37.85±0.04 ^e |
| Yeast and Fraction 7 | 36.02±0.19 ^a | 38.07±0.55 ^{de} | 38.18±0.37 ^c | 37.95±0.46 ^e | 37.99±0.55 ^e | 37.32±0.89 ^{ef} |
| Yeast and Fraction 8 | 36.76±0.27 ^{cd} | 37.97±0.03 ^{cde} | 37.38±0.20 ^b | 36.77±0.30 ^{bc} | 36.24±0.22 ^a | 36.04±0.25 ^{ab} |
| Yeast and Fraction 9 | 36.13±0.13 ^{abc} | 37.29±0.39 ^{bc} | 37.27±0.36 ^b | 37.12±0.23 ^{cd} | 36.86±0.28 ^b | 36.40±0.21 ^{abcd} |
| Yeast and Fraction 10 | 36.88±0.22 ^d | 38.19±0.07 ^e | 37.90±0.06 ^{bc} | 37.85±0.06 ^e | 37.33±0.06 ^{bcd} | 36.96±0.06 ^{cde} |
| Yeast and Fraction 11 | 36.64±0.10 ^{abcd} | 37.80±0.13 ^{cde} | 37.76±0.09 ^{bc} | 37.88±0.09 ^e | 37.79±0.03 ^e | 37.84±0.07 ^e |

Values are expressed as mean \pm standard error of mean. Mean values having common superscript letters in a column are not significantly different ($p < 0.05$) (using ANOVA followed by Duncan's multiple range test).

A wide range of chemicals found in medicinal plants are used in ancient systems of medicine, contemporary medications as well as nutraceuticals, dietary supplements, folk remedies, pharmaceutical intermediates, bioactive principles, and lead components in synthesized therapies (Rajasekaran, 2008). *K. senegalensis* is a medicinal plant that is extensively utilized in traditional systems of medicine and is considered to be one of the most significant. There are a number of compounds found in this plant, including flavonoids, phenols, tannins, saponins, alkaloids, terpenoids, steroids, and cardiac glycosides, that have been scientifically shown to have a variety of pharmacological characteristics. The results have revealed that phytochemicals may be extracted using a variety of solvents (Pimporn & Srikanjana, 2011), with the polarity of the solvent determining the extraction method.

In multimodal analgesia, the first-line treatment of pain and pyrexia is critical, and it is regarded to have a generally good safety profile, with the exception of severe overdose, and minimal medication interactions (Sharma & Mehta, 2013). Oral and rectal administration can produce analgesia within 40 minutes, with the maximum effect occurring at 1 hour. However, because of large variations in bioavailability (ranging from 63 to 89 percent for oral preparations and 24 to 98 percent for rectally administered preparations), the onset and duration of action can be unpredictable.

The antipyretic activity of the crude extract of *K. senegalensis* was found to be dose-dependent, which is similar to a study conducted by Swain et al. (2013) in which they assessed the analgesic and antipyretic activity of methanol extract of *Leucas clarki* in animal models and discovered that the higher concentration administered exhibited greater activity than the lower concentration administered (Swain et al., 2013, p. The presence of the phytochemicals found in the extracts of *K. senegalensis* may be responsible for the antipyretic action seen in the plant. *K. senegalensis* leaf extracts were found to contain tannins and alkaloids, which have antipyretic effects, according to Toma & Deyno (2014) who examined the phytochemistry and pharmacological activities of *Moringa oleifera* and concluded that tannins and alkaloids had antipyretic qualities.

It is necessary to evaluate the acute toxic potential of chemicals in order to identify the harmful consequences that may arise as a result of accidental or intentional short-term exposure to these substances. The LD₅₀ (median fatal dosage) of the studied extract of *K. senegalensis* leaf showed that the extract is reasonably safe at doses lower than 831.76mg/kg b.w. The extract is generally safe at doses lower than 831.76mg/kg b.w

Conclusion

The leaf methanol extract of *Khaya senegalensis* and its fractions possess significant antipyretic effect. Hence, the study has scientifically validated the traditional use of *K. senegalensis* leaf as antipyretic agent.

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