Analgesic and Anti-Pyretic Activities of the Root Bark of Rutidea Parviflora (Rubiaceae)

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Abstract

This study aims at investigating the antipyretic activity of different solvent fractions of the root bark of Rutidea parviflora (Rubiaceae). This plant is used ethno-botanically by the people of Ethiope East-West Local Government Area of Delta State, Nigeria to treat various ailments such as inflammation, fever and pain. This necessitated this research to validate its local use, due to the scanty literature and information present about this plant. It has also shown some anti-cancer and anti-inflammatory activity in previous researches. The present study is a randomized control study. Acetic acid induced writhing was employed for analgesic testing. Acetic acid was used to induce writhing in Wistar rats which were divided into fourteen (14) groups. The groups were administered extracts and fractions of the plant (200 mg/kg and 400 mg/kg). The animals were observed for number of writing movements and the percentage writhing was calculated. Baker’s yeast induced pyrexia was employed for the antipyretic testing. The animal groups were administered extracts and fractions of the plant (200 mg/kg and 400 mg/kg), with Paracetamol as the standard drug (100 mg/kg) and Normal saline (control) for both experiments. The body temperature of the rats was measured rectally over a period of five (5) hours. All values of P<0.05 were taken as significant. The organic extract, aqueous extract and various fractions (n-hexane, ethyl-acetate, n-butanol and aqueous) produced significant inhibition of writhing responses and pyrexia in a dose dependent manner and time dependent manner respectively. The aqueous extract at a dose of 400mg/kg showed the greatest reduction in writhing, 91.58% compared to the standard drug (paracetamol) which may suggest that the fraction possesses better efficacy than paracetamol as an analgesic. The observed activities could be attributed to these bioactive compounds: Palmatine, Urs-12-ene-24-oic-3-oxo-methyl ester and Gallic acid contained in R. parviflora.

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Introduction

Rubiaceae consist of various flowering plants called the madder family, bedstraw family or coffee family. The Rubiaceae family has 630 genera and almost 13000 species found worldwide in tropical and warm regions.

Many Rubiaceae family plants exhibit antimalarial, antimicrobial, anti-hypertension, anti-diabetic, antioxidant and anti-inflammatory activities. Bioactive compounds such as anthraquinones, alkaloids, indole and terpenoids have been isolated from these plants.

Two important genera of Rubiaceae with demonstrated anti-inflammatory activities include Borreria and spermacoce. The documented bioactivities of their isolated compounds include anti-inflammatory, anti-tumor anti-microbial, larvicidal, anti-oxidant, gastrointestinal, anti-ulcer and hepato-protective effects. Recently, Preliminary in vivo investigations of Borreria verticillata Linn (Rubiaceae) for analgesic and anti-inflammatory effects carried out indicated that the plant possessed significant (P>0.001) analgesic and anti-inflammatory activities at a dose range of 200 to 1000 mg/kg p.o/i.p in all models used.

Another related specie; Nauclea latifolia Smith (Rubiaceae) a small tree, native to Africa and used in traditional medicine by several indigenous communities for the treatment of fever, malaria, pain, epilepsy and anxiety has been found to induce hypothermia with significant antipyretic effects in mice. Also significant antinociceptive activity was recorded in all analgesia animal models used.

Recently, Nauclea latifolia was investigated for its anti-pyretic, anti-nociceptive and anti-inflammatory activities in two animal models. The researchers’ documented significant outcomes in all the activities evaluated in a dose-dependent manner.

There are scanty reports on the activities of R. parviflora. One study has documented the anti-cancer activity of this plant; R. parviflora. The evaluation of the cytotoxic activities of the compounds isolated from the R. parviflora plant demonstrated palmatine as the most potent bioactive compound. The anti-cancer activities of palmatine have been reported from several studies. Further investigations of the cytotoxic activities of palmatine in apoptosis assays demonstrated that the compound was cytotoxic to ovarian cancer cells.

R. parviflora is used by the indigenous people of Ethiope East/West Local Government in Delta State, Nigeria. Based on its ethno-medical use by the indigenous people in the treatment of inflammation alongside its associated fever and pain, this research became necessary to validate the ethno-medical use of this plant for analgesic and antipyretic effects beneficial in the treatment of pain and fever.

Materials and Method

Materials

Collection, Identification and Authentication

R. parviflora (root bark) was sourced from a bio reserve in Nigeria. The plant was authenticated by a botanist, Mr. Alfred O. Ozioko (INTERCEDD) with expert advice offered by Prof. J.F Bamidele of the Department of Plant Biology and Biotechnology, University of Benin, Nigeria. The voucher specimen numbers of the plant was; Rutidea parviflora INTERCEDD/1588.

Equipment and Instruments

Electronic weighing balance (model WT6002A), Maceration jars, Thermostat bath (HH-6; Techmel and Techmel, USA), Lypholiser (Harvest right scientific freeze dryer), Beakers, Glass funnels, Measuring cylinders, Conical flask, Rotary evaporator (R-205), Desiccator, Spatula, Crucibles, Filter papers, Syringes and Digital thermometers.

Reagents

Methanol, Dichloromethane, n-hexane, Ethyl acetate and n-butanol of Analytical grade (Sigma-Aldrich). Distilled water was obtained from the Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Port Harcourt.

Methods

Extraction of the Plant Materials

The plant materials were extracted according to the American National Cancer Institute (NCI) method of extraction. Typically, 200g of the pulverized plant...
material was macerated in a 1:1 mixture of 500ml of dichloromethane and 500ml of methanol for 24 h. The ratio of plant material to solvent used was 1:5. This ratio was maintained for all weighed amount of plant materials used. The obtained solution containing the extracts was decanted off and 500ml of methanol was added to the residue and allowed to stand for another 24 h. The solution of the extract was collected by filtration and 1 liter of deionized water was added to the residue. The aqueous extract was collected after 24 h of maceration. The methanol extraction was combined with the 1:1 dichloromethane and methanol extraction to yield the organic extract. This extraction solution was evaporated to dryness on a rotary evaporator at a temperature of 40°C. The obtained dry extracts were further dried in a desiccator to remove any trace of solvent. The aqueous extraction was dried using a lyophilizer to obtain a solid sample.

The percentage yields of the crude extracts of the plant were calculated as follows:

\[ \text{Percentage yield} = \frac{\text{Weight of Extract (g)}}{\text{Weight of Pulverized Powder (g)}} \times 100\% \]

**Sequential Fractionation of the Plant**

The organic extracts of the plant (about 22g) with the potent activity was subsequently partitioned based on increasing polarity in n-hexane, ethyl-acetate, and n-butanol consecutively. The extract was first dissolved in 90% methanol in water and partitioned with n-hexane three times (100ml x 3). The combined hexane extract was evaporated to yield the n-hexane fraction. The 90% methanol fraction was evaporated and the resulting residue was dissolved in water and partitioned with ethyl acetate three times (100ml x 3), which upon evaporation, the ethyl acetate fraction was obtained. Finally, partitioning in n-butanol (100ml x 3) was carried out. After separation, collection and evaporation of the solvents, the n-butanol and aqueous fractions was obtained. These four (4) fractions were investigated for anti-pyretic and analgesic activity.

**Phytochemical Screening**

The plant extract (crude extract of the root bark) was subjected to preliminary analysis using the method described by Trease and Evans 10. The results of the phytochemical screening of *R. parviflora* have been reported by our research team 11.

**Experimental Design**

This study was designed in line with the ethically approved experimental protocols adopted by the department of Experimental Pharmacology and Toxicology, of the Faculty of Pharmaceutical Sciences, University of Port Harcourt. Healthy adult Wistar albino rats irrespective of sex, weighing between 170-200 grams were selected for the study. These animals were obtained from the animal house of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Enugu, Nigeria. The animals were exposed to 12 hours of dark light cycle and kept under room temperature and humidity. After the animals were selected for study, they were separated in cages and were given food and water. The animals were allowed to acclimatize to laboratory conditions for 14 days prior to the experiments.

**Antipyretic Activity**

**Baker’s Yeast Induced Hyperthermia in Rats**

The method established by Tomazetti et al, 2005 12, with some modifications was employed in the antipyretic activity evaluation, with fever induced by Brewer’s yeast in rats. The basal rectal temperature of each rat was recorded at zero hour using clinical digital thermometer. Pyrexia was induced by subcutaneous injection of 15% w/v suspension of Brewer’s yeast in distilled water at a dose of 10 ml/kg body weight. In order to ensure uniform spreading of the suspension beneath the skin, the injection site was massaged. Immediately after yeast administration, food was withdrawn but access to water was still maintained. After 18 hours of Brewer’s yeast injection the rise in rectal temperature was recorded and only animals showing an increase in temperature of at least 0.6°C (or 1°F) were selected for the study. The mean increment recorded was 0.96°C after 18 h of administration. The animals were randomly divided into 14 groups, each group containing five rats. Group I received normal saline orally. Group II was given standard drug Paracetamol at the dose of 100 mg/kg per-oral.

The remaining Groups were Treated Orally as Follows;

- Groups III and IV received organic extract of the plant (*R. parviflora*) at oral dose of 200 mg/kg and 400 mg/kg respectively.
• Groups V and VI received aqueous extract at oral dose of 200 mg/kg and 400 mg/kg respectively.
• Groups VII and VIII received n-hexane fraction at oral dose of 200 mg/kg and 400 mg/kg respectively.
• Groups IX and X received ethyl acetate fraction at a dose of 200 mg/kg and 400 mg/kg po respectively.
• Groups XI and XII received n-butanol fraction at a dose of 200 mg/kg and 400 mg/kg po respectively.
• Groups XIII and XIV received aqueous fraction at oral dose of 200 mg/kg and 400 mg/kg respectively.

After the treatment, the temperature of all the rats in each group was recorded at 0, 1, 2, 3, 4 and 5 hours.

Analgesic Activity

Acetic Acid Induced Writhing Test

The method described by Koster et al., 1959, was used for the evaluation of analgesic activity in rats. The experimental animals were weighed and randomly divided into 14 groups consisting of 5 rats in each. Group I (control) received normal saline (10 ml/kg) orally. Group II (positive control) received standard drug Paracetamol at oral dose of 100 mg/kg. Remaining groups were treated orally as follows:
• Groups III and IV received organic extract at doses of 200 and 400 mg/kg respectively.
• Groups V and VI received aqueous extracts at doses of 200 and 400 mg/kg respectively.
• Groups VII and VIII received n-Hexane fractions at doses of 200 and 400 mg/kg respectively.
• Groups IX and X received ethyl acetate fractions at doses of 200 and 400 mg/kg respectively.
• Groups XI and XII received n-Butanol fractions at doses of 200 and 400 mg/kg respectively.
• Groups XIII and XIV received aqueous fractions at doses of 200 and 400 mg/kg respectively.

All treatments were administered orally. 45 minutes after administration of standard drug and test samples, each mouse was injected with 0.7% acetic acid at the dose of 10 ml/kg body weight intraperitoneally. The number of writhing responses manifested by each mouse was recorded for 30 minutes commencing just 5 minutes after acetic acid injection. The percentage analgesic activity was calculated as follows:

\[ \% \text{ inhibition of writhing} = \frac{W_c - W_t}{W_c} \times 100 \]

Where \( W \) is number of writhing, \( W_c \) is control, and \( W_t \) is test.

Statistical Analysis

All values were expressed as the mean ± standard error of the mean (SEM) and the results were analyzed statistically by one-way analysis of variance (ANOVA) for analgesic activity and multivariate analysis of variance (MANOVA) for antipyretic effect through time followed by Dunnet’s post hoc multiple comparison test by using SPSS ver. 16. For MANOVA, Levene’s test of equality errors of variance was performed. \( P < 0.05 \) was considered to be statistically significant.

Results

Extraction

Weight of pulverized powder = 1300g

Percentage yield = \( \frac{\text{Weight of Extract}}{\text{Weight of Pulverized Powder}} \times 100\% \)

Fractionation

Weight of Organic Extract used for fractioning = 22g

Percentage yield of fraction = \( \frac{\text{Weight of Fraction}}{\text{Weight of Organic Extract Used}} \times 100\% \)

The results in table 1, showed the percentage yield of the extracts and the fractions. It could be seen that the root bark of \( R. \) parviflora yielded less than 2% of the organic extract. This is not unexpected as roots tend to yield far less extracts than leaves or aerial parts of plants.

The phytochemical screening results are contained in Table 2. The findings include the presence of alkaloids, flavonoids, tannins, saponins and triterpenoids. There is documented evidence that plants with a combination of secondary metabolites such as alkaloids, flavonoids and saponins possess significant analgesic activities \(^{14, 15}\). Similarly, alkaloids, steroids, tannins and terpenoids are predominant inhibitors of
### Table 1. Yield and Percentage Yield of Extracts and Fractions

| EXTRACTS/FRACTIONS                  | YIELD (g) | PERCENTAGE YIELD (%) |
|-------------------------------------|-----------|----------------------|
| Organic Extract (Methanol/Dichloromethane) | 24        | 1.85                 |
| Aqueous Extract                     | 3.9       | 0.3                  |
| N-Hexane Fraction                   | 2.1       | 9.55                 |
| Ethyl Acetate Fraction              | 3.3       | 15                   |
| N-Butanol Fraction                  | 1.7       | 7.73                 |
| Aqueous Fraction                    | 1.15      | 5.23                 |

### Table 2. Result for Phytochemical Screening

|   | ALKALOIDS TEST                  | FLAVONOIDS TEST                  | TANNINS                  | CARBOHYDRATE TEST                  | SAPONIN TEST                  | PHLOBATANNINS                  | GLYCOSIDE TEST                  | TRITERPENOIDS                  |
|---|---------------------------------|----------------------------------|--------------------------|-----------------------------------|---------------------------------|---------------------------------|---------------------------------|-------------------------------|
| 1 | (a) Meyers test                 | (a) Shinoda test                 | (a) Ferric chloride test  | (a) Molish test                   | (a) Frothing test               | (a) Hydrochloride acid test     | (a) Keller killiani test       | (a) Leiberman-buchard test     |
|   | +ve                             | +ve                              | +ve                      | +ve                               | +ve                             | -ve                             | +ve                             | +ve                           |
|   | (b) Dragendorff test            | (b) Sodium hydroxide test        |                          | (b) Fehling’s test                |                                |                                 |                                 |                               |
|   | +ve                             | +ve                              |                          | +ve                               |                                 |                                 |                                 |                               |
|   | (c) Hagers test                 |                                  |                          |                                   |                                 |                                 |                                 |                               |
|   | +ve                             |                                  |                          |                                   |                                 |                                 |                                 |                               |
| 2 | FLAVONOIDS TEST                 |                                  |                          |                                   |                                 |                                 |                                 |                               |
|   |                                 |                                  |                          |                                   |                                 |                                 |                                 |                               |
| 3 | TANNINS                         |                                  |                          |                                   |                                 |                                 |                                 |                               |
|   |                                 |                                  |                          |                                   |                                 |                                 |                                 |                               |
| 4 | CARBOHYDRATE TEST               |                                  |                          |                                   |                                 |                                 |                                 |                               |
|   |                                 |                                  |                          |                                   |                                 |                                 |                                 |                               |
| 5 | SAPONIN TEST                    |                                  |                          |                                   |                                 |                                 |                                 |                               |
|   |                                 |                                  |                          |                                   |                                 |                                 |                                 |                               |
| 6 | PHLOBATANNINS                   |                                  |                          |                                   |                                 |                                 |                                 |                               |
|   |                                 |                                  |                          |                                   |                                 |                                 |                                 |                               |
| 7 | GLYCOSIDE TEST                  |                                  |                          |                                   |                                 |                                 |                                 |                               |
|   |                                 |                                  |                          |                                   |                                 |                                 |                                 |                               |
| 8 | TRITERPENOIDS                   |                                  |                          |                                   |                                 |                                 |                                 |                               |
|   |                                 |                                  |                          |                                   |                                 |                                 |                                 |                               |
|   |                                 |                                  |                          |                                   |                                 |                                 |                                 |                               |

**Key:** +ve = Present, -ve = Absent
Prostaglandin synthases; suggestive of anti-pyretic activities 16, 17.

Palmatine a quaternary protoberberine alkaloid that was previously isolated from Rhizoma coptidis, an important medicinal plant commonly used in the Traditional Chinese Medicine, TCM 18, has previously being isolated from R. parviflora. Also the presence of Urs-12-ene-24-oic-3-oxo-methyl ester; a pentacyclic triterpenoid and Gallic acid have been documented in R. parviflora 5.

Antipyretic Test Result

The results of the anti-pyretic evaluation of the extracts and fractions of R. parviflora are presented in Table 3 and figure 1 above. The results showed a rapid onset of activity by the 400 mg/kg doses of the extracts and fractions that were comparable to the standard drug PCM. The aqueous extract yielded very significant activity (P<0.05) than PCM; thus displaying superior anti-pyretic activity against PCM at 1 H. The extracts and fractions demonstrated significant effect on rectal temperature with significant reduction of temperature over a period of 3-5 H.

Further evaluation of the anti-pyretic activities was the extracts/fractions were carried out by calculating the percentage reduction of pyrexia as seen in figure 2 below.

A break-down of the percentage reduction of pyrexia in the rats displayed in figure 2 was in consonance with the observations made in Table 1 and Figure 1 previously; that the extracts and fractions had their maximum % reduction between 3-5 H; an indication that the herbal extracts and fractions had long lasting activity. The results at 1-2 H of the 400 mg/kg doses of the extracts/fractions and PCM showed that the activities were comparable. Also, the aqueous extract had a very significant activity (P>0.05) at 1 H, which surpassed that of PCM and the other extracts/fractions. But at the 3rd and 4th hour respectively PCM significantly inhibited pyrexia with the maximum % reduction being observed at the 4th hour of the experiment.

The analgesic effects of the extracts/fractions of R. parviflora were investigated and the results obtained are presented in Table 4, Figure 3 and 4 respectively.

Analgesic Test Result

The results of the analgesic experiments carried out are displayed in Table 4, and figures 3 and 4. There are strong indications that R. parviflora possess significant analgesic activities. This is evident from the dose-dependent and time-dependent significant activities recorded for the extracts and fractions. The analgesic result again validates the anti-pyretic activities of the extracts and fractions. It could be observed that the 400 mg/kg doses were comparable or superior to PCM. These results clearly support the indigenous use of this plant in the treatment of inflammations and fevers. Previous studies have linked the presence of alkaloids, flavonoids, tannins, terpenoids, saponins and steroids with good anti-pyretic activities. Saponins are known to inhibit the enzymes involved in the formation of pyrexia, while flavonoids hinders the synthesis of PG2 by inhibiting tumour necrosis factor –α responsible for the induction of fever. 16, 17

Discussion

The ethno-medicinal use of plants for the treatment of various ailments including microbial infections, neurological conditions, inflammation, pain and fever is common in Nigeria. The result of the phytochemical screening suggests that flavonoids and saponins are present which may explain this plant's antipyretic and analgesic activities. Other secondary metabolites present in the plant include alkaloids, tannins and glycosides. Numerous studies conducted on medicinal plants have associated the presence of metabolites such as flavonoids and alkaloids to anti-inflammatory, analgesic and antipyretic properties 16. Flavonoids are proposed to reduce arachidonic acid release through inhibition of neutrophils degranulation leading to the suppression of prostaglandins and leuotrienes responsible for inflammation, pain, and fever.

The extracts and different fractions from the root bark of R. parviflora (Rubiaceae) were assessed for analgesic activity against acetic acid induced writhing which is an indicator of visceral pain. The specific pain activity generated by intra-peritoneal injection of acetic acid is indicated with contraction of abdominal muscle associated with the stretching of hind limbs, elongation of the abdomen and other similar movements which are
Table 3. Antipyretic Effect of Extracts from Root Bark of *Rutidea parviflora* (Rubiaceae) and Its Fractions on Yeast Induced Pyrexia in Wistar Rats.

| Variables                  | Rectal Temperature (°C) | 0hr (after 18hr) | 1hr | 2hr | 3hr | 4hr | 5hr |
|----------------------------|-------------------------|-----------------|-----|-----|-----|-----|-----|
| Control                    | 37.8 ± 0.30a            | 39.05 ± 0.55b   | 38.50 ± 1.3b | 37.95 ± 1.65a | 37.00 ± 0.5a | 36.75 ± 1.48a | 36.15 ± 1.25a |
| Paracetamol (100mg/kg)     | 37.75 ± 0.30a           | 38.90 ± 0.10a   | 37.65 ± 0.75a | 36.60 ± 0.50a | 35.30 ± 0.00ac | 35.0 ± 0.00ac | 35.70 ± 0.65a |
| Organic Crude (200mg/kg)   | 38.50 ± 0.40b           | 39.20 ± 0.00a   | 38.10 ± 0.30b | 37.20 ± 0.50a | 36.10 ± 0.20a | 35.9 ± 0.45ac | 35.70 ± 0.20a |
| Organic Crude (400mg/kg)   | 35.05 ± 0.25a           | 35.70 ± 0.00a   | 37.05 ± 0.07a | 36.95 ± 0.25ac | 36.1 ± 0.25ac | 36.3 ± 0.65a | 36.45 ± 0.35a |
| Aqueous Crude (200mg/kg)   | 36.55 ± 0.25a           | 37.75 ± 0.15ac  | 37.05 ± 0.45a | 36.60 ± 0.50a | 35.95 ± 1.05c | 35.90 ± 0.8ac | 35.80 ± 0.10a |
| Aqueous Crude (400mg/kg)   | 35.90 ± 0.20a           | 36.75 ± 0.45ac  | 36.10 ± 0.40ac | 36.10 ± 0.5ac | 35.75 ± 1.15c | 35.75 ± 0.15ac | 35.65 ± 0.55a |
| n-Hexane Fraction (200mg/mg)| 37.60 ± 0.1a           | 38.15 ± 0.25a   | 38.05 ± 0.15ac | 37.55 ± 0.15ac | 36.9 ± 0.1ac | 36.6 ± 0.05a | 35.95 ± 0.15a |
| n-Hexane Fraction (400mg/mg)| 37.1 ± 0.6a            | 37.7 ± 0.6a     | 36.5 ± 0.4a   | 35.7 ± 0.3a | 35.55 ± 0.25a | 35.4 ± 0.3a | 36.10 ± 1.2a |
| Ethyl Acetate Fraction (200mg/kg) | 37.65 ± 0.05a | 38.45 ± 0.25a | 37.35 ± 0.15a | 37.2 ± 0.3ac | 36.8 ± 0.1c | 36.7 ± 1.2c | 35.55 ± 0.75a |
| Ethyl Acetate Fraction (400mg/kg) | 36.5 ± 0.7a | 37.05 ± 0.75 | 36.45 ± 0.85a | 36.30 ± 0.3a | 35.65 ± 0.85ac | 35.55 ± 0.65a | 35.55 ± 0.75b |
| n-Butanol Fraction (200mg/kg) | 38.05 ± 0.55b | 39.10 ± 0.10b | 37.8 ± 0.00a | 37.40 ± 1.6a | 36.65 ± 0.45a | 36.55 ± 0.25c | 36.45 ± 0.55b |
| n-Butanol Fraction (400mg/kg) | 36.95 ± 0.35a | 37.6 ± 0.05a | 37.15 ± 0.25a | 36.88 ± 0.55ac | 36.75 ± 0.25ac | 36.50 ± 0.6a | 36.45 ± 0.35b |
| Aqueous Fraction (200mg/kg) | 37.95 ± 0.55a | 38.6 ± 0.15a | 38.20 ± 0.10b | 37.90 ± 0.4a | 37.40 ± 0.9a | 37.00 ± 0.3b | 36.95 ± 0.15b |
| Aqueous Fraction (400mg/kg) | 37.35 ± 0.15a | 38.25 ± 0.05a | 36.75 ± 1.35a | 36.60 ± 0.9ac | 36.00 ± 0.95c | 35.90 ± 1.8ac | 35.45 ± 1.45a |

Footnote: Groups with different superscript indicate a significant difference at \( p < 0.05 \). All values represent mean± standard Mean Error.
Table 4. Analgesic Effect of Extracts from Root Bark of *Rutidea parviflora* (Rubiaceae) and Its Fractions on Acetic Acid Induced Writhing Test.

| Variables                          | Acetic Acid Induced Writhing Test (Analgesic Test) | % writhing |
|------------------------------------|---------------------------------------------------|------------|
| Control                            | 94.50 ± 0.85^a                                    | 0          |
| Paracetamol (PCM) (100mg/kg)       | 15.05 ± 0.44^b                                   | 84.21      |
| Organic Extract (200mg/kg)         | 77.03 ± 0.48^a                                   | 17.89      |
| Organic Extract (400mg/kg)         | 12.89 ± 0.24^b                                   | 86.32      |
| Aqueous Extract (200mg/kg)         | 52.75 ± 0.84^{ac}                                | 44.21      |
| Aqueous Extract (400mg/kg)         | 7.96 ± 0.83^b                                    | 91.58      |
| n-Hexane Fraction (200kg/mg)       | 18.09 ± 0.29^b                                   | 81.05      |
| n-Hexane Fraction (400kg/mg)       | 11.75 ± 0.41^b                                   | 87.37      |
| Ethyl Acetate Fraction (200mg/kg)  | 16.07 ± 0.41^b                                   | 83.16      |
| Ethyl Acetate Fraction (400mg/kg)  | 13.09 ± 0.71^b                                   | 85.26      |
| n-Butanol Fraction (200mg/kg)      | 48.91 ± 0.28^c                                   | 48.42      |
| n-Butanol Fraction (400mg/kg)      | 8.97 ± 0.41^b                                    | 90.53      |
| Aqueous Fraction (200mg/kg)        | 33.98 ± 0.82^c                                   | 64.21      |
| Aqueous Fraction (400mg/kg)        | 11.06 ± 0.35^b                                   | 88.42      |

Footnote: Groups with different superscript indicate a significant difference at $p < 0.05$. All values represent mean ± standard Error of Mean.
Figure 1. A Line Graph Showing the Antipyretic Effect of the Extracts of the Root Bark of *Rutidea parviflora* (Rubiaceae) and Its Fractions on Yeast Induced Pyrexia

![Line Graph](image)

Figure 2. A Bar Chart Showing the % Reduction of Pyrexia in Rats after administration of the Extracts of the Root Bark of *Rutidea parviflora* (Rubiaceae) and Its Fractions on Yeast Induced Pyrexia

![Bar Chart](image)
Figure 3. Line Graph for Analgesic Effects of Extracts of the Root Bark of *Rutidea parviflora* (Rubiaceae) and its Fractions on Acetic Acid Induced Writhing Test

Figure 4. Bar Chart for Analgesic Effects of Extracts of the Root Bark of *Rutidea parviflora* (Rubiaceae) and Its Fractions on Acetic Acid Induced Writhing Test
assumed to be mediated by local peritoneal receptor. The acetic acid triggered writhing is an economic, common and easy method for testing analgesic drugs. Acetic acid administration is responsible for the release of endogenous substances which excite the nerve endings, thereby causing the pain. Numerous researches have revealed the buildup of higher levels of prostaglandins (such as PGE2 and PGF2α), lipoygenase products (such as leukotrienes), and resident mast cells, in fluids treated with acetic acid and increase in pain sensation through capillary permeability.

The main impact of prostaglandins in producing pain response is mostly due to interaction with endogenous substances such as histamine, bradykinin, and substance P which further stimulate the sensitization of pain receptors to these mediators. NSAIDs relieve pain by inhibiting peripherally, the production of endogenous substances such as prostaglandins, thromboxane, and other inflammatory mediators by acting on cyclooxygenase enzymes (COX 1 and COX 2). Any substance that reduces the number of abdominal constrictions induced by acetic acid can be considered to have analgesic activity.

The extracts and fractions of R. parviflora significantly reduced the number of writhing in dose dependent mode. The extracts and fractions at the dose of 200 mg/kg and 400 mg/kg produced significant reductions in the number of writhing (P < 0.05) produced by acetic acid in rats when compared to the control group.

The aqueous extract at a dose of 400 mg/kg showed the greatest reduction in writhing, 91.58% (P< 0.05) compared to the standard drug (paracetamol) which showed a reduction in writhing of 84.21% and the other samples. However, the organic fraction at a dose of 200 mg/kg showed an almost insignificant reduction in writhing of 17.89% but produced significantly better analgesia at a dose of 400mg/kg which was 86.32% (P< 0.05). This strongly suggests that the plant under study possesses good peripheral analgesic property, possibly elicited by a similar mechanism of action to the standard drug (paracetamol) through the inhibition of prostaglandins, thereby decreasing inflammation along with its associated pain and fever.

The extracts and different fractions from the root bark of R. parviflora (Rubiaceae) were also assessed for antipyretic activity against yeast induced fever which is an indicator of pathogenic fever. The yeast induced fever is an economic, common and easy method for testing antipyretic drugs. The proteins present in yeast induce fever by stimulation of inflammation. Furthermore, the production of endogenous pyrogens such as pro-inflammatory cytokines (interleukin [IL-1β and IL-6], interferons [IFN-α] and tumor necrosis factors [TNF-α]) and prostaglandins (PGE2 and PG12) are responsible for increasing the temperature of the body by acting on the hypothalamus in the brain.

Antipyretics such as paracetamol employed for use in management of pyrexia have several mechanisms of action such as reducing prostaglandins levels by acting on cyclooxygenase (COX) enzymes, enhancing hypothalamus thermo-regulatory activities and managing anti-inflammatory signals such as inflammatory cells and molecules.

The subcutaneous injection of yeast markedly increased the rectal temperature and the mean increment recorded was 0.96°C after 18 h of administration. The different treatment extracts/fractions of the plant and paracetamol lowered the rectal temperature in time dependent manner. Several researches carried out by numerous researchers have shown that medicinal plants showing anti-inflammatory activity may also possess antipyretic and analgesic activities as the mechanism of action elicited for the suppression of inflammation, fever, and pain can be linked to the inhibition of mediators of inflammation.

Previous studies by our research team have obtained palmatine and urs-12-ene-24-oic acid, 3-oxo-methyl ester from R. parviflora. While gallic acid was identified from the GCMS determination of the organic extract of R. parviflora. Palmatine an anti-cancer agent; has been documented to possess anti-inflammatory and anti-pyretic activities amongst other activities. Previous researchers have documented the anti-asthma, anti-arthritis, anti-inflammatory, anti-microbial and diuretic activities.
of Urs-12-ene-24-oic acid, 3-oxo-methyl ester\(^{36}\).

Recently, the anti-pyretic and analgesic activities of gallic acid was reported from an investigation of the anti-oxidant, anti-inflammatory, analgesic and antipyretic activities of grapevine leaf extract (\textit{Vitis vinifera}) in mice. LC–MS/MS analyses revealed the presence of anthocyanin, catechin, gallic acid, resveratrol, quercetin, flavone, flavonols and epicatechin as the active constituents of the plant\(^ {37}\).

**Conclusion**

The extracts and different fractions from the root bark of \textit{R. parviflora} (Rubiaceae) displayed significant (\(P < 0.05\)) analgesic and antipyretic properties of equal potency when compared to the standard drug paracetamol. The aqueous extract at a dose of 400 mg/kg showed the greatest reduction in writhing, 91.58% compared to the standard drug (paracetamol) which may suggest that it possesses better efficacy than paracetamol as an analgesic.

The precise mechanisms involved in the production of the antipyretic and analgesic activities of the plant may be caused by the presence of the palmatine, urs-12-ene-24-oic acid, 3-oxo-methyl ester and gallic acid in the root-bark of \textit{R. parviflora} (Rubiaceae).

**Conflict of Interest**

There is no conflict of interest disclosed.

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