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Full Length Research Paper

Phytochemical and analgesic evaluation of *Mondia whytei* (hook.f) root

GITHINJI, C. G.[1]*, P. M. MBUGUA[2], T. I. KANUI [3] AND D. K. KARIUKI[4],

[1]Department of Medical Physiology, School of Medicine, College of Health Sciences, University of Nairobi, P. O. Box 30197-00100, Kenya
[2]School of Health Sciences, Presbyterian University of East Africa. P. O. Box 387-00902, Kikuyu, Kenya.
[3]Department of Range and Wildlife Sciences, School of Agriculture and Veterinary Sciences, South Eastern College University, P. O. Box 170 – 90200, Kitui, Kenya.
[4]Department of Chemistry, School of Biological and Physical Sciences, University of Nairobi, P. O. Box 30197-00100, Nairobi, Kenya.

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***Mondia whytei* is a forest floor plant that belongs to *Asclepidaceae* family. The roots are widely used in African traditional medicine to treat various diseases and conditions that include pain, swelling, postpartum hemorrhage, diabetes mellitus among many other claims. Phytochemical tests were carried out to determine the different chemical constituents of the root skeels of *M. whytei.* Analgesic activity of the root powder was evaluated using acetic acid induced writhing test. Phytochemical screening revealed the presence of carotenoids, flavonoids, steroids and tannins. The root skeels significantly ($p < 0.05$) reduced the number of writhes in acetic acid induced writhing test. This reduction was dose-dependent. Therefore, the use of the plant by traditional medical practitioners in the treatment of pain may be justified.**

Key words: *Mondia whytei,* roots, phytochemical, writhing test, analgesic.

INTRODUCTION

Mondia whytei is a forest floor plant with aromatic rhizomatous roots that belongs to the A*sclepiadaceae* family. *Asclepiadaceous* has more than 300 genera and 2000 species (Van Heerden and Steyn, 1999). However, there are about 104 species of the family that have been identified in East Africa (Agnew and Agnew, 1994).

The *Asclepiadaceae* family is mostly found in the tropics and subtropical regions. *M. whytei* is distributed widely in Africa. It is found in Guinea in West Africa through Sudan, Uganda, Kenya, Tanzania, Malawi, South Africa and Westwards to Angola (Beentje, 1994).

In Kenya, *M. whytei* is locally known as Mukombela (Luhya), Ogomba (Luo), Olkonkola (Maasai), Mkonkora (Kamba) and Muhukura (Kikuyu). Outside Kenya, the plant is known as Omurondwa (lunyore-Uganda), Ilivi (sudan), Omondi (Zulu) and Mbombogazi (Tanzania) (Kokwaro, 2006).

The roots are chewed or boiled with porridge to treat post partum pains, gastrointestinal colic and dysmenorrheal (Jain et al., 1996). It is also claimed to have anti-inflammatory, anti-pyretics and antimicrobial activities (Jain et al., 1996). It is also used as an aphrodisiac (Kokwaro, 2006). Parasympathomimetic effects of *M. whytei* aqueous root extract on rabbit heart and jejunum is well documented (Githinji et al*.,* 2007).

There is need to develop analgesic drugs devoid of side effects associated with opiods and non steroidal anti inflammatory agents. Research on plants like *M. whytei* with claims of having analgesic activity is therefore important in developing novel analgesic drugs. The aim of this study was to evaluate the phytochemical groups

*Corresponding author. E-mail: ggithua@uonbi.ac.ke. Tel: +254 (20) 722 785 517.

responsible for analgesic activities of *M. whytei* root.

MATERIALS AND METHODS

Harvesting of *M. whytei* roots

Plant materials of *M. whytei* were collected from Kakamega forest, Western province of Kenya during the flowering stage in August 2006 and dried under shade. The plant material was identified in the Department of Botany, University of Nairobi, Kenya. Voucher Specimen no. CG/MV/608 was deposited at the herbarium of Department of Botany, Univeristy of Nairobi, Kenya.

The skeels of the roots were peeled off when fresh and allowed to dry for three weeks under a shade. One kilogram of the root skeels was pulverized using a Molly grinder and the powder stored in a glass container at room temperature awaiting extraction and phytochemical tests.

Extraction of *M. whytei* roots powder with chloroform, ethanol and water

One hundrend grams (100 g) of the root powder were extracted with chloroform, ethanol and water using Soxhlet apparatus for 48 hours at 60 to 80°C. Fresh powder was used in each extraction. The extract was ultrafiltered using sintered glass connected to Butchner funnel and under sunction pressure. The filtrate was then reduced to minimum volume using rotary vacuum evaporator and allowed to dry under room temperature. Water extracts were freeze dried. The three extracts were each subjected to acetic acid induced writhing test.

Defatting of *M. whytei* roots powder with n-hexane prior to successive extraction with chloroform and methanol

One hundrend grams of the root powder were extracted with n-hexane (defatting) using Soxhlet apparatus for 48 h at 60 to 80°C. The marc was then successively re-extracted with chloroform and then methanol in the Soxhlet apparatus for 48 h. Each of the extract was reduced to minimum volume using rotary vacuum evaporator and allowed to dry under room temperature. The three extracts were each subjected to acetic acid induced writhing test.

Screening for various phytochemical groups in the roots

Spot tests for alkaloids were carried out according to the method described by Sofowara (1993). One gram of the root powder was extracted by heating it on a water bath with 10 ml of 10% sulphuric acid for 5 min. It was filtered and a portion of the extract tested for alkaloids by adding 2 drops of Mayer's reagent. The presence of alkaloids was indicated by formation of white precipitates.

Tests for saponins was carried out according to the method described by Evans (1996). Two grams of the root powder were boiled in 20 ml of distilled water on a water bath and then filtered. Ten milliliters of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. Three drops of olive oil were added and the mixture shaken vigorously, then observed for the formation of emulsion.

The test for flavonoids was carried out according to the method described by Geissman (1995). Five milliliters of dilute ammonia solution was added to a portion of the aqueous filtrate of the root powder. This was followed by addition of concerntrated sulphuric acid. Yellow colouration indicated the presence of flavonoids.

The test for total anthraquinones (bound and free) and phenolics was carried out according to the method described by Harbone (1973). Half gram of the root powder was boiled with 5 ml dilute sulphuric acid for 5 min. It was filtered while hot and upon cooling the filtrate was added with an equal volume of carbon tetrachloride and vigorously mixed. A rose pink to red colour in the ammoniacal layer indicated presence of anthraquinones and phenolics. Chloroform root extracts were subjected to a test for carotenoids (Trease and Evans, 1989). One gram of each extract was boiled in 20 ml water. The filtrate was then treated with concentrated sulphuric acid. Blue colour indicated the presence of carotenoids.

The root powder was subjected to modified Stas Otto extraction of glycosides and the extract subjected to Kedde's test for unsaturated lactone ring/cardenolide and Keller-Killian's test for a 2-deoxy sugar (Sim, 1967). One gram of the root powder was extracted with 10 ml of 70% alcohol by heating on a water bath for 2 min and then cooled and filtered. Ten milliliters of water and 5 drops of lead subacetate were added to the filtrate to form a precipitate. It was filtered and 10% sulphuric acid added drop wise until no further precipitate formed. The precipitates were filtered and extracted with two successive 5 ml portions of chloroform. The extracts were combined and washed with 1 ml distilled water. For the Kedde's test, one portion of the chloroform extract was evaporated to dryness; one drop of 90% alcohol and 2% 3, 5-dinitrobenzoic acid in 90% alcohol were added and then made alkaline with 20% sodium hydroxide solution. A purple reaction colour with 3, 5-dinitrobenzoic acid indicated a positive result. Keller-Killan test was carried out by evaporating the chloroform portion to dryness and adding 0.4 ml glacial acetic acid containing trace of ferric chloride. It was then transferred to a test tube and 0.5 ml of concerntrated sulphuric acid added carefully along the side of the test tube. A green-blue colour in the upper acetic acid layer confirmed a positive test.

Thin Layer Chromatography (TLC) evaluation for saponins, flavonoids, and alkaloids was carried out according to the methods described by Wagner and Bladt (1996). The presence for steriodal nucleus was detected by TLC as described by Obdoni and Ochuko (2001). Two milliliters of acetic anhydride was added to 0.5 g ethanolic extracts of each sample. The presence of steroids was indicated by colour change from violet to blue or green.

Experimental animals

Sixty male and female Swiss albino mice weighing 25 to 30 g were used. The animals were housed in colony cages with free access to food and water and allowed to acclimatize for one week. They were kept in rooms with temperature of 23 $\pm$ 1.0°C and relative humidity of 50%. Diurnal rhythms was regulated with a 12 h light: 12 h dark cycle with lights on 7.00 a.m to 7.00 p.m. The animals were handled humanely in accordance with the institutional animal welfare committee guidelines.

Experimental design

Animals were randomly picked up by touch and pick technique and assigned to an experimental unit based on the type of test extract being evaluated. Each experimental unit comprised a treated group and control group.

In all experiments each animal was used only once. The test materials were prepared and appropriate dilutions made in clearly labeled sample bottles. The samples were then coded by an independent person to ensure blinding. The coded samples were

Table 1. Percentage yield from the bark, leaves and roots of *M. whytei* using chloroform, ethanol and water.

Extract	% yield
Aqueous (water) Root - (AR)	15.0
Ethanol Root - (ER)	44.3
Chloroform root - (CR)	28.0

Table 2. Effects of increasing doses of *M. whytei* water, ethanol and chloroform root extracts on abdominal writhes.

Dose (ml/kg)	Aqueous root (AR)		Ethanol root (ER)		Chloroform root (CR)	
	Mean ± S.E.M	% Inhibition	Mean ± S.E.M	% Inhibition	Mean ± S.E.M	% Inhibition
Control saline 10	90.5±2.37	(-)	88.8±5.51	(-)	87.0±2.87	(-)
25	81.8±3.12***	9.6	80.9±3.41*	8.8	74.1±3.31***	14.8
50	74.0±3.02***	18.2	80.2±4.10*	9.6	66.0±3.43***	24.1
Extract 100	61.1±2.28***	32.4	76.0±3.40***	14.4	52.2±3.49***	40
200	58.7±3.02***	35.1	62.4±4.84***	29.7	43.2±3.19***	50.3
400	49.5±2.51***	45.3	57.0±7.24***	35.8	33.2±2.27***	61.8

* p< 0.05 and ***p< 0.001; n = 6.

thereafter given to the researcher who administered them as per the codes. The codes were broken after data analysis.

Drug administration

All injections were given intraperitoneally in volume of 10 ml/kg. Aspiration prior to injection was performed to ensure that the drug was not injected into the intestines. In all experiments an equal volume of vehicle was used as control. The drugs were injected 30 min prior to pain assessment.

Acetic acid induced writhing test

Two hours before testing, the animals were placed individually in an observation chamber made of perspex box (30 x 30 x 30 cm). The animals did not have access to water and food two hours prior to the experiment. With a minimum restraint, 0.1 ml/10 g body weight of 0.7 v/v acetic acid was administered intraperitoneally.

Immediately after the injection of the acetic acid, each animal was isolated and placed in a box (30 x 30 x 30 cm) for observation. The numbers of writhes were counted over a period of thirty min, recorded and expressed as the percentage protection of writhing using the following ratio: (control mean-treated mean) x 100/control mean.

Statistical analysis

Data was analyzed using analysis of variance (ANOVA). When the analysis was restricted to two means, the student's t-test (paired and one tailed) was used. Scheffe' post hoc test was done for multiple comparisons. The level of significance was set at p<0.05. Results are presented as mean ± standard error of the mean (s.e.m). The absolute values of the number of writhes and the time spent in pain behaviors as recorded were used in statistical calculation.

Statistical Package for Social Sciences (SPSS) version 16.0 statistical package was used for data analysis.

RESULTS

Yield of the water, ethanol and chloroform root extract

The highest yield was that of ethanol root (ER) extract which was 44.3% of the extracted root powder (Table 1).

Effects of water, ethanol and chloroform root extracts in abdominal writhing

Aqueous root (AR), ethanol root (ER) and chloroform root (CR) extracts significantly ($p < 0.001$) and dose dependently reduced the number of abdominal writhes (Table 2). However, Chloroform root extracts caused higher inhibitory effects on abdominal writhes compared to the other two extracts (Table 2). The dose that lowered the number of abdominal writhes by half compared to the control (ID_{50}) for CR extract was 198.8 mg/kg (Figure 1).

Yield from the defatted root powder

The root powder was first extracted exhaustively with n-hexane (defatting) and then successively with chloroform and methanol. Hexane root (HR) extract yielded 18.0% of the extracted root powder. It was an oily mass. Chloroform (CR_d) and methanol (MR_d) extracts yielded

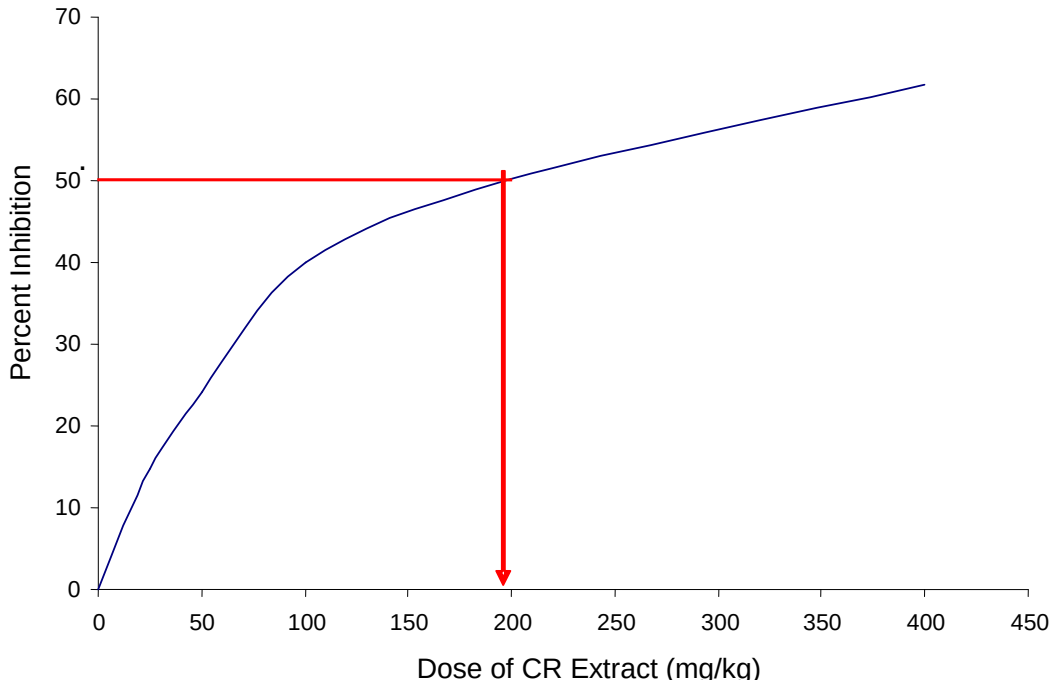

Figure 1. Dose response curve for chloroform root extract. (CR). The ID_{50} is 198.9 mg/kg.

Table 3. Percentage yields of the defatted root powder.

Extract			% Yield
Hexane Root	-	(HR)	18.0
Defatted Chloroform Root	-	(CR_d)	18.3
Defatted Methanol root	-	(MR_d)	48.0

18.3 and 48.0% of the extracted root powder. Both extracts were pasty in consistency (Table 3).

Effects of the defatted *M. whytei* n-hexane, chloroform and methanol root extracts on abdominal writhing

Table 4 shows the effects of various doses of the defatted *M. whytei* root extracts on the number of abdominal writhes. Hexane root (HR), defatted methanol root (MR_d) and defatted chloroform root (CR_d) extracts induced significant ($p < 0.001$) reduction in the number of abdominal writhes at all dose levels compared to the control. This effect was dose dependent.

Figure 2 is the bar graph of the effects of various extracts at varied doses. It reveals defatted chloroform root extracts (CR_d) having higher inhibitory effects on abdominal writhes compared to the other extracts. The effects of CR_d extracts were significantly ($p < 0.01$) higher at all dose levels tested compared to HR extracts. MR_d extracts had effects that were significantly ($p < 0.01$) different from HR extracts except at a dose of 50 mg/kg.

From the dose response curve (Figure 3) the ID_{50} for CR_d was 350 mg/kg. The effects of this dose was statistically significant ($p < 0.001$) compared with the control saline. HR and MR_d could not achieve their ID_{50} at the the highest dose tested that is 400 mg/kg (Figure 3). Defatting the extract prior to antinociceptive testing caused the ID_{50} of CR to rise from 198.8 to 350 mg/kg. This suggested wide solubility range of the active compounds between n-hexane and chloroform.

Phytochemical groups detected in the crude root powder and the chloroform root extract of *M. whytei*

Preliminary investigation by TLC and spot tests revealed anthraquinones, cardenolides, carotenoids, flavonoids, steroids, tannins and 2-Deoxy sugars in the whole root powder.

Chloroform extracts when subjected to phytochemical

Table 4. Effects of increasing doses of defatted *M. whytei* root extracts on abdominal writhes.

Dose (ml/kg)		N-Hexane root extract (HR)		Defatted chloroform root (CRd)		Defatted methanol root (MRd)	
		Mean ± S.E.M	% Inhibition	Mean ± S.E.M	% Inhibition	Mean ± S.E.M	% Inhibition
Control saline 10		89.3 ± 2.07	(-)	85.2 ± 3.31	(-)	89.5 ± 2.88	(-)
	25	84.8 ± 1.94*	5	80.0 ± 1.41*	6.1	81.2 ± 1.94***	9.3
	50	76.8 ± 2.23***	14	69.0 ± 0.26***	19	74.7 ± 1.97***	16.5
Extract	100	72.7 ± 2.07***	18.6	61.0 ± 2.10***	28.4	63.2 ± 2.14***	29.4
	200	65.8 ± 1.47***	26.3	50.3 ± 2.07***	41	60.0 ± 1.41***	33
	400	60.2 ± 1.94***	32.6	39.8 ± 1.83***	53.3	49.0 ± 2.37***	45.3

* p< 0.05 and ***p< 0.001; n = 6.

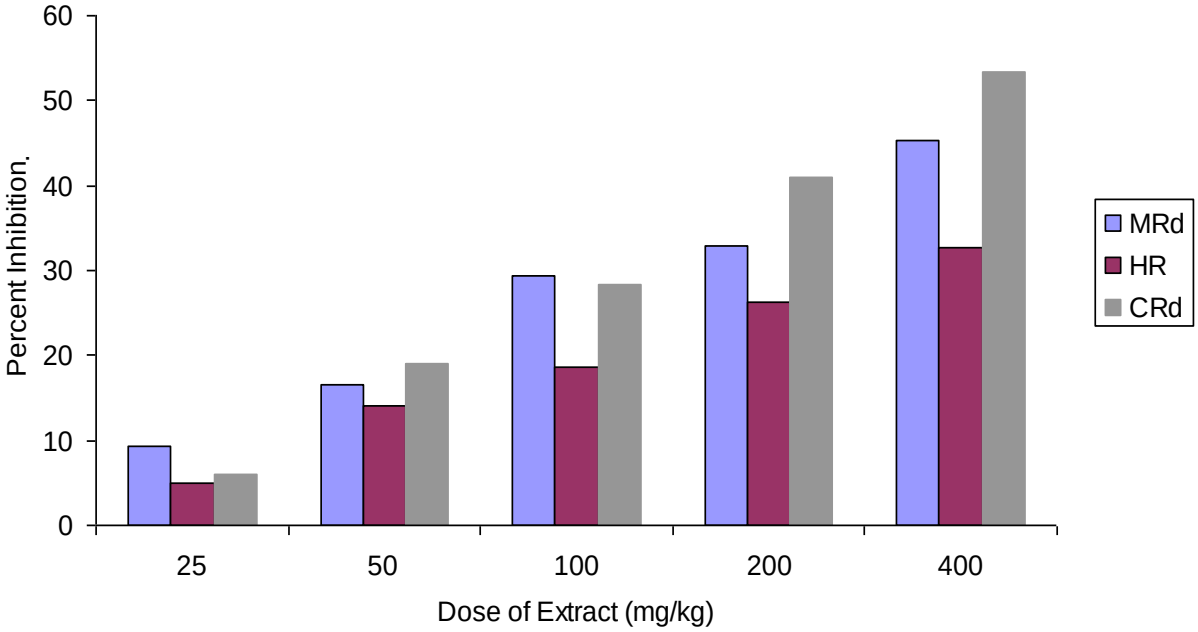

Figure 2. Effects of Intraperitoneally Administered MR_d, HR and CR_d Extracts on the Number of Abdominal Writhes. ** p< 0.01 and *** p< 0.001; n = 6.

tests were shown to contain carotenoids, steroids, flavonoids and tannins (Table 5).

DISCUSSION

Acetic acid induced writhing test in mice was used to screen *M. whytei* for antinociceptive activity. This test is commonly used as a screening method because it is easy to perform and is sensitive (Taber, 1974). The main disadvantage of this method is its lack of specificity as many drugs without analgesic effects in man can effectively inhibit writhing responses (Chernorv et al., 1967). In addition, there is a large variation in response between mouse strains (Finn et al., 1997). Adult male and female Swiss albino mice weighing 25 to 30 mg were used. Equal number of male and female was assigned to each experimental unit. This countered variation of result attributed to gender differences. Acclimatization of the animals to the experimental environment was done for two weeks before subjecting them to the tests. Also adaptation of the animals to the testing chamber was done two hours prior to testing. This had an overall effect of reducing stress to the animal which would otherwise led to variations in the results obtained since stress could result in stress induced analgesia.

Chloroform root (CR) extracts caused the highest inhibition of the abdominal writhes compared to the ethanol and water extracts (Table 2). These findings suggested that the active compounds responsible for

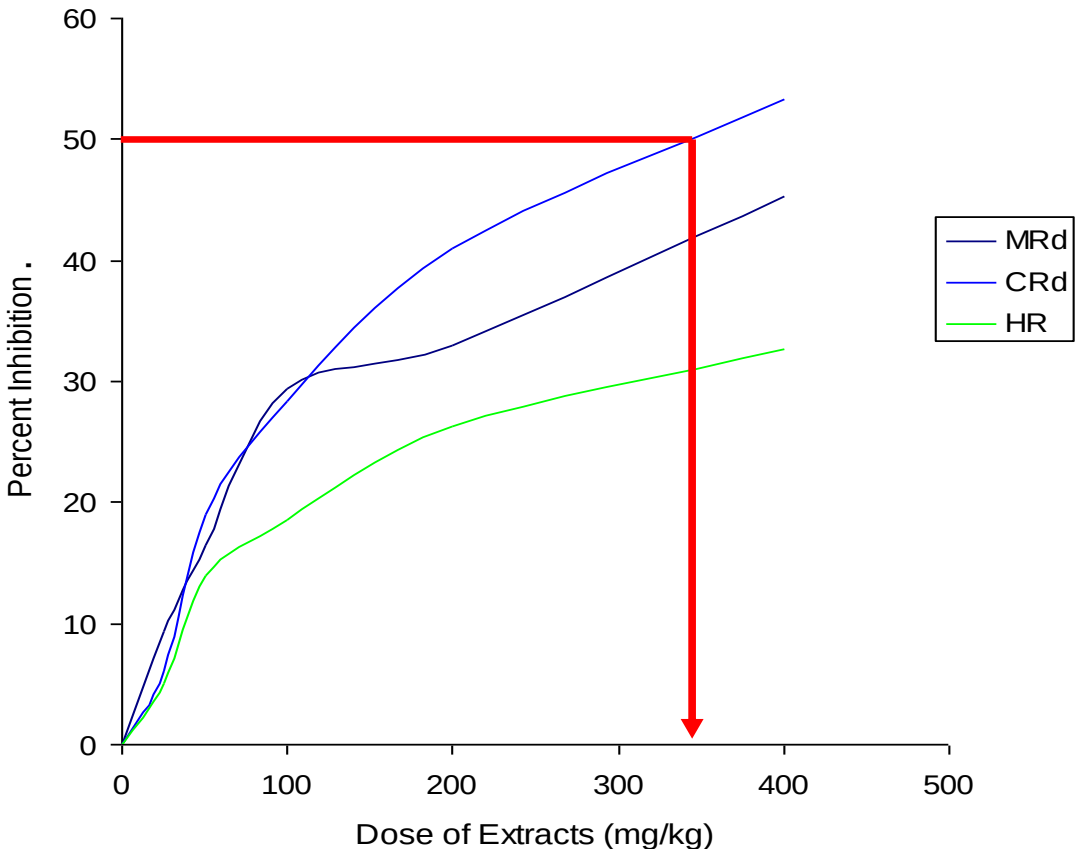

Figure 3. Dose Response Curves of CR_d, MR_d and HR. The ID_{50} is 350 mg/kg for CR_d.

Table 5. Phytochemical groups detected in the crude root powder and the chloroform root extracts of *M. whytei.*

Phytochemical group	Crude root powder	Chloroform root extract
Alkaloids	Absent	Absent
Anthraquinones Glycosides	Present	Absent
Cardenolides	Present	Absent
Carotenoids	Present	Present
Flavanoids	Present	Present
Steroids	Present	Present
Saponins	Absent	Absent
Tannins	Present	Present
2-Deoxy Sugar	Present	Absent

antinociceptive activities of *M. whytei* are non polar in nature. Phytochemical tests done on the chloroform root extract confirmed the presence of carotenoids, flavonoids, steroids and tannins. CR extracts also contained plenty of plant oils. However, plant oils are known to have low pharmacological activity (Peter and Amala, 1998). CR had an ID_{50} of 198.8 mg/kg, the dose that lowered the number of abdominal writhes by half compared to the control. This was statistically significant ($p < 0.01$) whereas ethanol and water root extracts could not achieve ID_{50} at the highest dose tested (400 mg/kg).

Defatting of the root powder with n-hexane prior to extracting with chloroform was meant to remove plant oils and therefore lower the ID_{50} of the defatted chloroform root extract (CR_d). On the contrary, the ID_{50} of CR_d was raised to 350 mg/kg (Figure 3). This meant that n-hexane dissolved some of the active compounds prior to extracting with chloroform. This suggested a wide solubility range of the active compound between n-hexane and chloroform.

Conclusion

In conclusion, chloroform root extract revealed the most potent analgesic activity in acetic acid induced writhing test. Carotenoids, flavonoids, steroids and tannins were the phytochemical groups found in the chloroform root extract. Steroids and flavonoids have been shown to have analgesic effects (Garcia et al., 1999). The use of *M. whytei* roots as a pain killer in traditional herbal medicine may therefore be justified.

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