Evaluation of Phytochemical Screening and Analgesic Activity of Aqueous Extract of the Leaves of Microtrichia perotitii Dc (Asteraceae) in Mice using Hotplate Method

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Abstract
Currently, available drugs for the management of pains, fever and inflammation conditions present with it many known adverse effects, hence the search for new drugs from plants which hitherto may be harmless to humans. To this end, Microtrichia perotitii (Asteraceae) was screened for its phyto contents and analgesic properties using hotplate method with mice. The result of the preliminary Phytochemical studies revealed the presence of tannins, flavonoids, saponins, alkaloids, carbohydrates and phenolics in the plant as a whole. The analgesic study showed that the aqueous extract of the leaves have significant activity (P<0.05; P<0.001) as compared to morphine sulphate (10 mg/kg) used as a standard drug. The result indicated dose-independent activity. Tannins, flavonoids, alkaloids and saponins have been reported to be responsible for the analgesic and anti-inflammatory activities in many medicinal plants of this family. These results may explain the use of the plant for the management of pains and its related ailments in the locality where it is very common.

Keywords
Microtrichia perotitii; Asteraceae; Analgesic properties; Hotplate method; Phytochemical studies; Morphine sulphate

Background
Pain as a sensation is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage according to International Association for the study of Pain (IASP) or described in terms of such damage (Merskey, 1986; Michael et al., 2003). Pain therefore is a perception similar to vision and hearing and is a symptom that cannot be objectively assessed (Angel, 2009). Pain is a discomfort whose relief must be tackled all round. Drugs mostly used for the management of pains are either opioids which are commonly used for the management of acute postoperative pain or non-opioids and these drugs have been reported to possess potential toxic effect such as gastrointestinal bleedings (Ambarkar et al., 2011). On the other hand drugs of plant origin have been used for management of diseases for many centuries and have not been reported of any deleterious effects to their hosts. It is for this reason that Microtrichia perotitii DC (Asteraceae) which is a shrub was selected for this study (Watson and Dallwitz, 1992). The plant is widely distributed in West Africa. In Nigeria it is cultivated in North Nigeria around Zaria province which extends up to Birnin Gwari (in Kaduna State).

In other parts of West Africa it is common in Senegal, Quassadous, Mali, Port of Guinea, Sieraleone, Ivory coast, Ghana and Dahomey (Hutchinson and Dalziel, 1963).

In trado-medical application, the leaves of the plant are chewed to treat toothaches and its related ailments. Similarly, cold concoction of the leaves is used as mouthwash to prevent the teeth from decay. On the other hand sap squeezed from the fresh leaves is used to treat rashes in children while the entire plant is used spiritually to dispel evil spirits in some communities.

In addition, this plant is almost going into extinction in many communities and there are no much reported work on its potentials most especially the active components it contains.
1 Results

1.1 Phytochemical Screening

The following components were identified in the crude aqueous extract of the leaves of Microtrichia perotitii (Table 1).

Table 1 Some identified Phytochemical constituents from the crude aqueous extract of the leaves of Microtrichia perotitii

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Tests</th>
<th>Aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>Lead acetate</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Ammonia Solution</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Ferric Chloride</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Shinoda test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Ferric chloride</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Lead acetate</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Sodium hydroxide</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Dragendoff’s reagent</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Wagner reagent</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Mayer reagent</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrate (reducing sugars)</td>
<td>Molich test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Fehlings’ solution</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosiates</td>
<td>Keller-kilian (cardenoides)</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Salkowskts test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Liberman-burchard test</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>Frothing test</td>
<td>+</td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>1% HCl</td>
<td>–</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>Borntrager’s test</td>
<td>–</td>
</tr>
<tr>
<td>Phenolics</td>
<td>Ferric chloride</td>
<td>+</td>
</tr>
<tr>
<td>Resins</td>
<td>10% KMnO₄</td>
<td>–</td>
</tr>
</tbody>
</table>

Note: +: detected; -: absent

1.2 Analgesic activity (Table 2; Figure 1)

![Graph showing analgesic activity](image)

Figure 1 Effect of aqueous crude extract of M. perotitii in mice when exposed to a Hot-plate

2 Discussion

The preliminary Phytochemical screening of the aqueous extract of the leaves of Microtrichia perotitii gave positive test for carbohydrates, cardiac glycosides, alkaloids, flavonoids, saponins, tannins and phenolics which could account for the antinociceptive activity of the plant as reported in other plants (Hemmalini et al., 2011; Jain et al., 2011). Similar compounds were reported for some members of the Asteraceae family that exhibited analgesic properties (Saritha et al., 2012). Flavonoids compounds are known to target prostaglandins which are involved in the late phase of acute inflammation and pain perception, hence its presence in the aqueous extract of the plant may be contributory to the analgesic effects of the plant (Rao et al., 1998; Rajnarayana et al., 2001). There are also reports on the role of tannins in anti-nociceptive activity (Vanu et al., 2006).
Table 2 Analgesic activity of crude Aqueous Extract of the Leaf Microtrichia perotitii using Hotplate method

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>Time interval (mins)</th>
<th>Aqueous crude Extract</th>
<th>Mean latency±SD</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline (-ve control)</td>
<td>10</td>
<td>0</td>
<td>1.44±0.16</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>1.35±0.21</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>1.51±0.19</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>1.23±0.52</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>25</td>
<td>0</td>
<td>1.67±0.21</td>
<td>1.10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>1.50±0.22</td>
<td>1.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>1.97±0.23*</td>
<td>3.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>1.68±0.19*</td>
<td>3.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>0</td>
<td>1.35±0.21</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>1.11±0.23**</td>
<td>1.76</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>1.27±0.18**</td>
<td>1.78</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>1.35±0.11</td>
<td>0.87</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>0</td>
<td>1.50±0.16</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>1.00±0.23*</td>
<td>3.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>1.09±0.15*</td>
<td>3.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>1.16±0.17</td>
<td>0.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morphine sulphate (+ve control)</td>
<td>4</td>
<td>0</td>
<td>1.33±0.15</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>1.44±0.06**</td>
<td>1.54</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>1.29±0.09**</td>
<td>1.63</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>1.35±0.12</td>
<td>0.87</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: *significant at p<0.05; ** significant at p<0.001

On the other hand alkaloids are well known for their effects to inhibit pain perception (Uche et al., 2008, Amritpal et al., 2008).

The analgesic effect of a substance can either be induced via central nervous system or the peripheral nervous system or both in some rare cases. Therefore, it is always necessary to employ a method to distinguish between the two (Tjolsen et al., 1992; Raval and Ravishankar, 2010). Animal tests of analgesics drugs commonly measure nociception and involve testing the reaction of an animal to painful stimuli (Rang et al., 2003). However, in this study the hotplate method was used to establish the effectiveness of the extract in evaluating the central nervous system analgesia. In this test the extract somehow increased the mice reaction on the hotplate and the difference between the mean reaction time and then time of treated groups and the control indicates dose-independent activity. The hot plate response is more complex supraspinally organized behaviour (Mustaffa et al., 2010; Chapman et al., 1985).

The result of the antinociceptive activity of Microtrichia perotitii is presented in Table 2 & fig 1. The result showed that there was no significant difference in the pain reaction time (PRT) before drug administration in all the mice. However, 30 mins after administration the PRT was significantly (p<0.05 and p<0.001) increased by the extract and the reference drug (Morphine sulphate) but in a dose-independent manner when compared to the normal saline treated group. At doses of 25 mg/kg and 100 mg/kg the PRT was much higher than the standard drug used which compared relatively with the treatment at 50 mg/kg. Invariably in this experiment the extracts at 25 mg/kg
and 100 mg/kg were more effective than the standard drug (Pavan et al., 2009; Omeh and Ezeja, 2010). Increase in the pain reaction time (latency period) indicates the level of analgesia induced by the drug and extracts. Increase in stress tolerance capacity of the animals indicates the possible involvement of higher centre (Omeh and Ezeja, 2010). Therefore, the overall result obtained suggested that the aqueous extract of the leaves of Microtrichia perotitii had antinociceptive activity in the hotplate test that may be in part mediated by opioid receptors and therefore can relieve pain which equally justify the use of the plant for toothaches (Barau et al., 2009).

3 Materials and methods
3.1 Experimental Animals
Adult Swiss albino mice and Wister rats of both sexes and body weight were obtained from the animal house of the Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University Zaria and used for this research work. They were kept in well-ventilated condition and fed with standard excel feeds plc (Kaduna) grower mash. The animals were allowed access to food and water *ad libitum*. The animals were handled well in compliance with the National Regulations for Animal Research which is in conformity with the regulations of Ahmadu Bello University Animal Research and ethics guidelines.

3.2 Collection and Identification of Microtrichia perotitii DC
Fresh herbs of Microtrichia perotitii DC were collected during the matured and flowering stages of the plant in April 2005 (Figure 2). The plant was collected from the swampy areas in Rigasa village a sub-urb of Kaduna metropolis in Kaduna state. The plant was scientifically identified from its botanical and taxonomic characteristics. (Daniel, 1877; Andrews, 1954; Hutchinson and Dalziel, 1963; Watson and Dallwitz, 1992). Authentic sample was prepared and deposited at the Herbarium of the Department of Biological sciences, Ahmadu Bello University, Zaria and given a batch number 998 by the Herbarium officer mal. Musa M.

3.3 Extraction of M. perotitii DC
300 g each of the powdered leaf material was extracted first with Petroleum ether (60~80°C) and the marc extracted in distilled water using Soxhlet apparatus for 20 h. After the extraction period the solute was separated from the solvent by gradual distillation and later evaporated to dryness on boiling hot water bath. The extractable was allowed to cool and placed in a clean container and kept sealed in a desiccators until use (Brain and Turner, 1975; Ciulei, 1994; Wang and Weller, 2006).

3.4 Phytochemical screening
The crude aqueous extract of the leaves of Microtrichia perotitii was screened for its Phytochemical components using standard methods (Agarwal, 2000).

3.4.1 Test for tannins
3.4.1.1 Lead Acetate Test
To 2 mL each of the aqueous solution of the extracts, 2 mL of water was added in a test -tube followed by addition of 5 drops of lead acetate solution. The test-tube was shaken. Appearance of coloured precipitate indicates the presence of tannins (Kokate et al., 2002).

3.4.1.2 Ammonia Solution Test
0.2 g of the aqueous solution of the extract was transferred into a test-tube. To this was added 3 mL of water, shaken and then filtered. To the filtrate 3 mL of 25% ammonia solution was added and the test tube exposed to the air. Appearance of a slowly forming green colouration indicates the presence of chlorogenic acid (Evans, 2002; Kokate, 2002).
3.4.1.3 Ferric chloride Test
Small quantity of each extract was mixed with distilled water and heated on a boiling water bath. Each mixture was filtered and to the filtrate few drops of concentrated H$_2$SO$_4$ and 5% Ferric chloride solution were added. Appearance of a blue-black, green or blue-green precipitate indicates the presence of tannins (Evans, 2002).

3.4.2 Test for Flavonoids
3.4.2.1 Shinoda’s test
To few mls of the alcoholic extract, few pieces of Magnesium turnings were added followed by two drops of concentrated hydrochloric acid. Occurrence of effervescence with formation of dark brown solution which gradually changes to a deep red solution or appearance of pink colouration indicates the presence of flavonoids (Evans, 2002).

3.4.2.2 Ferric Chloride Test
About 5 mL of distilled water was added to each of the extract and boiled on hot water bath for 2 min in a test-tube. The content was filtered and to 2 mL of the filtrate few drops of 10% alcoholic ferric chloride solution was added. Occurrence of effervescence and the changing of the dark brown colour of the solution to green, blue to violet indicates the presence of flavonoids (Evans, 2002).

3.4.2.3 Lead Acetate Test
0.2 g of each crude extract was dissolved in water and then filtered. In a test-tube few drops of 10% lead acetate was added to 5 mL of the filtrate. Appearance of buff coloured precipitate indicates the presence of flavonoids (Brain and Turner, 1975; Harborire, 1998).

3.4.2.4 Sodium Hydroxide Test
To 2 mL of each extract in a test tube was added 10% of sodium hydroxide solution. Appearance of yellow colouration indicates the presence of flavonoids (Evans, 2002).

3.4.3 Test for Alkaloids
3 mL of each extract was stirred with 5 mL of 1% aqueous hydrochloric acid on hot water bath. The content was filtered and divided into 3 portion of 1 mL each in test-tubes. To the first portion was added few drops of Dragendorff’s reagent and occurrence of orange red precipitate indicates the presence of alkaloids, to the second portion was added few drops of Wagner reagent and occurrence of reddish brown colour indicates the presence of alkaloids while to the third portion few drops of Mayer reagent were added and appearance of buff precipitate indicates the presence of alkaloids (Evans, 2002).

3.4.4 Test for Carbohydrates
3.4.4.1 Molisch’s Test
0.2 g of each crude extract was dissolved in 10 mL of distilled water. To this solution in a test-tube few drops of Molisch’s reagent were added. This was followed by the addition of 1ml of concentrated H$_2$SO$_4$ gradually down the side of the test-tube so that the acid forms a layer beneath the aqueous layer. The mixture was allowed to stand for 2 min and then diluted with 5 mL of water. Formation of a red to dull violet colour at the interface of the two layers indicates positive test for general carbohydrates (Evans, 2002).

3.4.4.2 Fehling’s Test (Reducing Sugar)
Each of the plant extracts was hydrolysed by boiling with 5 mL dilute hydrochloric acid and the resulting solution was neutralized with sodium hydroxide solution. To each of the solutions few drops of Fehling’s A and B solutions were added and then heated on boiling water bath for 2 mins. Formation of reddish brown precipitate of cuprous oxide indicates the presence of carbohydrate (Evans, 2002).

3.4.5 Test for Cardiac Glycosides
3.4.5.1 Keller-Kiliani test for Cardenolides
0.5 g of each crude extract was dissolved in 2 mL of 3.5% Ferric chloride solution in glacial acetic acid in a test-tube. To this was added 2 mL of concentrated H$_2$SO$_4$. Occurrence of reddish-brown ring at the interphase indicates the presence of digitalis glycoside (Evans, 2002).

3.4.5.2 Salkowski’s Test
0.2 g of each crude extract was dissolved in 2 mL of Chloroform in a test-tube. To this was added carefully from the side concentration H$_2$SO$_4$ to form a lower layer. Occurrence of reddish-brown colour at the interface indicates the presence of steroidal nucleus (Sofowora, 1993)
3.4.5.3 Liberman-Burchard Test
0.2 g of each crude extract was dissolved in 2 mls of chloroform in a test-tube. Few drops of acetic anhydride were added to the tube and was boiled and cooled. Concentrated H$_2$SO$_4$ was added from the side of the test-tube using a pipette. Formation of a brown ring at the junction of the two layers and the turning of the upper layer to green shows the presence of steroids while formation of deep red colour indicates the presence of triterpenoids (Harbone, 1973; Culei, 1994).

3.4.6 Test for Saponins (Frothing Test)
0.5 g of each crude powder was shaken vigorously with 10ml of distilled water in a test-tube. The test-tube was warmed in a water bath for 5mins and later shaken for observance of frothing. The persistence of the froth indicates the presence of saponins. The frothing was mixed with 3 drops of olive oil and observed for the formation of emulsion which confirms the presence of saponins (Harbone, 1973; Kapoor et al., 1969; Sofowora, 1993).

3.4.7 Test for Phlobatannins
10 mL of aqueous extract of each sample was boiled with 1% HCl acid in a test-tube after filtration. Appearance or deposition of a red precipitate indicates the presence of phlobatannins (Sofowora, 1993; Evans, 2002).

3.4.8 Test for Anthraquinones
3.4.8.1 Borntrager’s Test
2 g of each powdered material was boiled with 4 mL of 10% HCl for 3min. the mixture was filtered while still hot and the filtrate allowed to cool. The cooled filtrate was then shaken with equal volume of chloroform to extract the anthraquinone. The chloroform layer was then transferred into a clean test-tube and treated with equal volume of 10% NH$_4$OH. The mixture was then shaken well and the colour of the upper layer noted. Appearance of colourless layer indicates the presence of anthraquinone i.e anthracene derivates (Evans, 2002).

3.4.8.2 Ammonium Hydroxide Test
0.5 g of each crude powder was shaken with 10ml of benzene and was filtered 2 mL of 10% NH$_4$OH was added to the filterate and the mixture further shaken well. Appearance of a pink, red or violet colouration in the ammoniacal layer (lower) indicates the presence of anthraquinone (Evans, 2002).

3.4.8.3 Test for Phenolic Compounds’ Iron III Chloride Test
To 2 mL each of the ethanolic extract of the powdered maternal was added 3 mls of water in a test-tube. To this was added 2 drops of 1% Ferric III chloride solution. Appearance of red, blue, green (blackish) or purple colour indicates the presence of phenolic compound (Evans, 2002).

3.4.8.4 Test for Resins
To 0.5 g of the extract, 5 mL of 10% KMnO$_4$ solution was added and then heated gently in a test-tube over Bunsen flame. Perception of odour of benzaldehyde due to oxidation of benzoic acid indicates the presence of resins (Brain and Turner, 1975; Evans, 2002).

3.4.9 Hot Plate Test
Thus test was carried out based on the method described by Eddy and Leimback (1953). The experimental animals of either sex were randomly selected and divided into five groups consisting of five mice each to serve as control, (positive control) and test sample group respectively. The first group (control group) received normal saline (0.9% w/v NaCl Solution), while groups 2, 3 and 4 received 25 mg, 50 mg and 100 mg extract/kg body weight p.o respectively, and group 5 received Morphine sulphate (positive control) at a dose of 10 mg/kg body weight p.o. The animals were positioned on a hot plate kept at a temperature of (55±0.5)°C. A cut off period of 15 seconds was observed to avoid damage to the paw. Reaction time was recorded when animals licked their fore or hind paws or jumped prior to and 30 min, 60 min and 90 min after oral administration of the samples. The maximum possible effect (MPE) was calculated as: %MPE=(test latency-control latency)/(cut off time- control latency)$\times$100 (Toma et al., 2003; Kulkarni, 1999; Heidari et al., 2007)

3.5 Statistical analysis
The results were analysed for statistical significance using ANOVA, followed by Scheffe’s test. $p<0.05$ and
p<0.001 values were considered significant. Computer statistical package SPSS (version 16) was used for the analysis.

Acknowledgement

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