

## Investigation of the Laxative Activity of *Operculina turpethum* Extract in Mice

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### ABSTRACT

The study was aimed to investigate the laxative activity of leaf extracts of *Operculina turpethum*. The dried leaves of *Operculina turpethum* was successively extracted with hexane, chloroform and 70% methanol using cold maceration method. The resulting extracts were concentrated in hot air oven at 40 °C. The laxative activity of *Operculina turpethum* leaves was investigated using *in vivo* models; faecal consistency, intestinal motility and enteropooling in mice. Two doses (200 and 400 mg/kg) of each extract was administered and castor oil was used as positive control. The treatment of the mice with the extracts and castor oil produced various degrees of wet faeces. The chloroform and methanol extract of *Operculina turpethum* produced a significant ( $P < 0.05$ ) dose- and time-dependent increase in the percentage of wet faeces in the treated groups when compared to the negative control group. The extract caused a significant ( $P < 0.05$ ) dose-dependent increase in the intestinal motility in the treated mice when compared to the negative control. The treatment of the mice with the extracts did not produce any significant ( $P > 0.05$ ) change in the intestinal content volume when compared to the negative control. In conclusion the *Operculina turpethum* extract showed a potent laxative activity and confirms its use for this purpose in traditional medicine.

**Keywords:** operculina turpethum, laxative activity, castor oil, charcoal meal

### INTRODUCTION

Herbal medicines are fast becoming popular even in this generation where everything is governed by science. Healing with medicinal plants predates written human history as archaeological evidence indicates that use of medicinal plants dates at least to the Palaeolithic, approximately 6,000 years ago<sup>1</sup>. Plant's medicinal value is due to the presence of phytochemicals that produce physiological and pharmacological actions in the body<sup>2</sup>. Medicinal plants may not have dosage instructions but they are known to have all-natural ingredients and a variety of other advantages including; lower cost than prescription medications, multi-systemic effect, reduced risk of side effects, widespread availability, cultural acceptance etc<sup>3</sup>. One of the commonly used medicinal plants in botanical medicine is *Operculina turpethum*. *Operculina turpethum* (Indian Jalap), a plant in the morning glory family (*Convolvulaceae*), is a perennial herbaceous plant with purplish stems and somewhat hairy vine reaching a length of 4 to 5 metres or more. Leaves are entire, alternate and variable in shape, narrowing to a pointed tip, broad and somewhat heart-shaped or straight at the base. Sepals are brittle and green<sup>4</sup>. *Operculina turpethum* is native to Asia, Africa and Australia while it is naturalised in the West Indies<sup>5</sup>. In many countries, *Operculina turpethum* is used as folk medicine to treat constipation, jaundice, rheumatism, haematemesis, tuberculosis, herpes, chronic

gout, piles, and tumours and induce laceration<sup>6,7</sup>. In the Philippines, Nepal and India, the root is used as a drastic purgative<sup>8</sup>. It is used topically to treat vitiligo and other skin disorders, alopecia, cervical lymphadenitis, haemorrhoids, fistulas, ulcers and chancres<sup>9,10</sup>. The leave is extensively used in Bende Abia State, Nigeria in the relief of constipation (Oral communication). The antioxidant, haematopoietic, hepatoprotective, antiulcer, antimicrobial and antidiabetic properties *Operculina turpethum* have been reported<sup>11-16</sup>. There is a paucity of scientific information on the laxative or purgative activity of *Operculina turpethum* despite the extensive use in ethnomedicine for this purpose. This study was designed to evaluate the laxative activity of leaf extracts of *Operculina turpethum*.

### MATERIALS AND METHODS

#### Collection and identification of the plant material

Fresh leaves of *Operculina turpethum* were collected from Ozuitem in Bende Local Government Area of Abia State, South Eastern Nigeria and were confirmed as *Operculina turpethum* by a plant taxonomist, Dr. M. C Dike of College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike. Sample catalogued MOUAU/VPP/2014/011 was deposited in the department of Veterinary Physiology, Pharmacology, Biochemistry and Animal Health and

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Production, College of Veterinary Medicine, Micheal Okpara University of Agriculture, herbarium.

#### Extraction of plant material

The leaves of *Operculina turpethum* were dried under room temperature on a laboratory bench, and were ground to coarse powder using manual grinder (Corona, China). The powdered material was weighed using an electronic balance. The plant material (150 g) was first macerated with hexane and then the marc was macerated with chloroform and 70% methanol successively for 48 h with intermittent shaking every 2 h. The respective extracts were filtered with Whatmann No. 1 filter paper and were dried in a hot air oven at 40 °C. The extract was stored in a refrigerator at 4 °C until time of use. The percentage yields (w/w) of the extracts were calculated using the formula below:

$$\left( \frac{\text{Weight of extract} + \text{weight of starting plant material}}{\text{weight of starting plant material}} \right) \times 100$$

#### Animals

One hundred and twenty (120) mice (28- 34 g), sourced from the laboratory animal unit of the Department of Veterinary Physiology, Pharmacology and Biochemistry, Michael Okpara University of Agriculture Umudike, Abia State were used for the study. The animals were housed in aluminium cages at room temperature and under natural light/darkness cycles. The mice were supplied with clean drinking water and fed *ad libitum* with standard commercial pelleted grower feed (Vital feed® Nigeria). The mice were acclimatized for two weeks prior to the study. They were maintained in accordance with the recommendations of the Guide for the care and use of laboratory animals<sup>17</sup> and the experimental protocol was approved by the institution's ethical committee.

#### Experimental procedures

##### Acute oral toxicity test

This was done using the up-and-down method<sup>18</sup>. Eight mice were randomly divided into four groups of two mice per group. Group A received 10 ml/kg of distilled water while groups B – D received 2000 mg/kg of hexane, chloroform and methanol extracts of *Operculina turpethum* each and the animals watched for 48 h for signs of toxicity or mortality. Dead animals were counted and percentage mortalities were calculated. After 24 hours, four mice were randomly divided into two groups of two and were treated respectively with 1500 mg/kg of Chloroform and methanol extracts of *Operculina turpethum*.

##### Effect of *Operculina turpethum* on faecal consistency

The modified method as described by Atta and Mouneir<sup>19</sup> was adopted for the study. Forty mice were divided randomly into eight groups of five mice each and were fasted for 16 hours before the experiment. Group A received 10 ml/kg of distilled water, group B received castor oil (0.3 ml/animal), groups C, E and G received 200 mg/kg of hexane, chloroform and methanol extracts of *Operculina turpethum* respectively while groups D, F and H received 400 mg/kg of hexane, chloroform and methanol extracts of *Operculina turpethum* respectively. The animals were kept in individual cages with the floors lined with blotting papers and the number of both normal and

watery droppings was counted every hour over a period of 4 hours. Mean number of the stools passed by the treated groups were compared with that of the control. The percentage wet faeces was calculated thus;

$$\left( \frac{\text{Mean number of wet faeces} + \text{Total number of faeces}}{\text{Total number of faeces}} \right) \times 100$$

##### Effect of *Operculina turpethum* on intestinal transit time

The method described by Vogel *et al*<sup>20</sup> was used in this experiment. Forty mice were randomly divided into eight groups of five mice each and were fasted for 16 hours before the experiment. Group A received 10 ml/kg of distilled water, group B received castor oil (0.3 ml/animal), groups C, E and G received 200 mg/kg of Hexane, Chloroform and Methanol extracts of *Operculina turpethum* respectively while groups D, F and H received respectively 400 mg/kg of Hexane, Chloroform and Methanol extracts of *Operculina turpethum*. One hour post treatment, standard charcoal meal was administered to all the animals. The animals were sacrificed 30 minutes post administration of charcoal meal by cervical dislocation and the intestines immediately isolated and ligated at the pyloric sphincter and ileocaecal junction. The small intestinal transit was expressed as percentage of distance travelled by the charcoal meal relative to the total length of the small intestine from the pyloric sphincter to the ileocaecal junction.

##### Effect of *Operculina turpethum* on enteropooling

The method of Hassan *et al*.<sup>21</sup> was used. Briefly, forty mice were randomly divided into eight groups of five mice each and were fasted for 16 hours before the experiment. Group A received 10 ml/kg of distilled water, group B received 0.3 ml castor oil per animal, groups C, E and G received 200 mg/kg of Hexane, Chloroform and Methanol extracts of *Operculina turpethum* respectively while groups D, F and H respectively received 400 mg/kg of *Operculina turpethum*. One hour post treatment, the animals were sacrificed by cervical dislocation, laparotomized and the intestines immediately isolated and ligated at the pyloric sphincter and ileocaecal junction. The small intestines were weighed, the content of each intestine was milked out, and the empty intestines were reweighed. The difference in weight between the full and empty intestines was recorded as the weight of the intestinal content.

##### Statistical analysis

The mean faecal output, small intestinal transit and volume of the accumulated fluid were analyzed using one way analysis of variance (ANOVA). The experimental results were expressed as mean ± standard error of mean (SEM) and significance difference was accepted at a probability level of  $p < 0.05$ .

## RESULTS

### Yield of the extract

The hexane leaf extract of *Operculina turpethum* yielded 0.93%w/w. The material was dark green in colour, pasty in consistency and has a leafy smell. The chloroform leaf extract yielded 13.87%w/w, which was brownish green in colour, pasty in consistency and has a pungent smell. The methanol extracts yielded 7.33%w/w, which was dark brown in colour, pasty in consistency and has a less

Table 1: Effect of *Operculina turpethum* on the percentage wet faeces in mice

Treatment	1 Hr	2 Hr	3 Hr	4 Hr
Distilled water 10 ml/kg	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Castor oil 0.3 ml/animal	32.74 ± 11.96*	60.28 ± 6.83*	68.48 ± 4.53*	74.94 ± 4.01*
Hexane 200 mg/kg	25.00 ± 19.36	11.90 ± 6.12	6.07 ± 2.80	10.26 ± 6.12
Hexane 400 mg/kg	10.00 ± 7.75	3.57 ± 2.77	8.33 ± 6.45	16.67 ± 7.45
Chloroform 200 mg/kg	0.00 ± 0.00	50.71 ± 9.57*	68.03 ± 6.74*	77.24 ± 5.38*
Chloroform 400 mg/kg	42.00 ± 15.58*	73.68 ± 5.47*	77.50 ± 2.24*	85.52 ± 0.86*
Methanol 200 mg/kg	0.00 ± 0.00	42.29 ± 14.33*	49.11 ± 16.47*	51.37 ± 17.14*
Methanol 400 mg/kg	5.00 ± 4.08	83.61 ± 4.74*	88.58 ± 3.71*	92.56 ± 1.98*

\*p < 0.05 when compared to the negative control

Table 2: Effect of the extracts on small intestinal transit in mice

Treatment	% intestinal transit
Distilled water 10 ml/kg	48.58 ± 3.95
Castor oil 0.3 ml/animal	49.53 ± 4.39
Hexane 200 mg/kg	61.74 ± 0.31*
Hexane 400 mg/kg	63.90 ± 3.19*
Chloroform 200 mg/kg	55.69 ± 2.18
Chloroform 400 mg/kg	67.58 ± 4.38*
Methanol 200 mg/kg	54.96 ± 4.68
Methanol 400 mg/kg	62.68 ± 2.61*

\*p < 0.05 when compared to the negative control

Table 3: The effect of the extracts on the accumulation of intestinal content in mice

Treatment	Volume (ml)
Distilled water 10 ml/kg	0.53 ± 0.19
Castor oil 0.3 ml/animal	0.30 ± 0.09
Hexane 200 mg/kg	0.40 ± 0.06
Hexane 400 mg/kg	0.38 ± 0.09
Chloroform 200 mg/kg	0.40 ± 0.04
Chloroform 400 mg/kg	0.50 ± 0.04
Methanol 200 mg/kg	0.40 ± 0.06
Methanol 400 mg/kg	0.48 ± 0.10

No significant difference (p > 0.05) when compared to the negative control  
pungent smell.

*Acute toxicity test*

No mortality was observed at the dose of 2000 mg/kg of the hexane extract of *Operculina turpethum* but the same dose produced 50% mortality in animals treated with the methanol extract and 100% mortality in animals treated with the chloroform extract.

At the dose of 1500 mg/kg, the chloroform extract produced 50% mortality in animals that were treated with chloroform extract while the animals that received the methanol extract had no mortality. Thus the median lethal doses (LD<sub>50</sub>) of the plant extracts were found to be 1500 mg/kg for the chloroform extract, 2000 mg/kg for the methanol extract and greater than 2000 mg/kg for the hexane extract.

*Effect of Operculina turpethum on faecal consistency*

The effects of *Operculina turpethum* in faecal consistency is presented in Table I. The treatment of the mice with the

extracts and castor oil produced various degrees of wet faeces. The chloroform and methanol extract of *Operculina turpethum* produced a significant (P < 0.05) dose- and time-dependent increase in the percentage of wet faeces in the treated groups when compared to the negative control group. The pre-diarrhea period of the chloroform extract (200 mg/kg) and methanol extract (200 mg/kg) was greater than 1 h but less than 2 h, while the pre-diarrhea period of hexane extract (400 mg/kg), chloroform extract (400 mg/kg), methanol extract (400 mg/kg) and castor oil was less than 1 h. The effects of the chloroform and methanol extracts were comparable to the effects produced by castor oil. The chloroform extract (400 mg/kg), methanol extract (400 mg/kg) and castor oil produced 85.52 %, 92.56% and 74.94% wet faeces at 4 h post treatment respectively.

*Effects of Operculina turpethum on intestinal motility*

The extract caused a significant (P < 0.05) dose-dependent increase in the intestinal motility in the treated mice when compared to the negative control (Table 2). The hexane extract (400 mg/kg), chloroform extract (400 mg/kg), methanol extract (400 mg/kg) and castor oil caused the charcoal meal to travel 63.90%, 67.58%, 62.68% and 49.53% of the total intestinal length respectively in the treated mice.

*Effects of Operculina turpethum on enteropooling*

The effects of *Operculina turpethum* on enteropooling are presented in Table 3. The treatment of the mice with the extracts did not produce any significant (P > 0.05) change in the intestinal content volume when compared to the negative control.

**DISCUSSION**

The laxative activity of *Operculina turpethum* leaves was investigated using in vivo models; faecal consistency, intestinal motility and enteropooling in mice. Laxatives are agents which enhance the evacuation of unformed watery faeces from the entire colon<sup>22</sup>. They act by: enhancing retention of intestinal fluid by hydrophilic or osmotic mechanism, decreasing net fluid absorption by effects on small- and large-intestinal fluid and electrolyte transport, and stimulation of intestinal motility<sup>23</sup>. The extracts of *Operculina turpethum* leaves demonstrated a potent cathartic activity through causing: discharge of watery faeces (Table 1), enhanced intestinal motility (Table 2) and increase in intestinal fluid content (Table 3), which were comparable to the effects produced by castor oil. This

suggests that the extracts of *Operculina turpethum* and castor oil may have similar mechanism of action. Castor oil increases intestinal fluid contents and causes diarrhea indirectly through ricinoleic acid formation, which changes the electrolyte and water transport and generates enormous contractions in the transverse and distal colon<sup>24</sup>. The cathartic effect of the plant extract may be mediated by the presence of some phytochemical component(s)<sup>25</sup>. The underlining mechanism of *Operculina turpethum* extract enhanced gastrointestinal motility and intestinal content is not known, but gastrointestinal motility is regulated by multiple physiological mediators, such as acetylcholine, histamine, substance P, cholecystokinins, prostaglandins and 5-hydroxytryptamine<sup>24, 26</sup>. The release of these chemicals in the gut causes a stimulatory effect mediated through an ultimate increase in cytosolic Calcium ion, and a substance with the ability to stimulate any of the above pathways is considered to be effective hyperactive gut stimulants<sup>27</sup>. The extracts may have sensitized cyclooxygenase (COX-1) leading to enhanced synthesis of prostaglandins in gastric mucosa. The prostaglandins stimulate mucus and biocarbonate secretion, decrease acid secretion and cause vasodilation. The action of prostaglandins on EP<sub>3</sub> receptors causes contraction of intestinal smooth muscle, inhibition of gastric acid secretion, increased mucus secretion, inhibition of lipolysis, inhibition of autonomic neurotransmitter release and stimulation of contraction of the pregnant human uterus<sup>23, 28, 29</sup>. Another possible mechanism of action of the *Operculina turpethum* extracts could be by activation of NO synthase and the biosynthesis of platelet-activating factor in the gut. Platelet-activating factor is a phospholipid proinflammatory mediator that stimulates colonic secretion and GI motility. Nitric oxide also may stimulate intestinal secretion and inhibit segmenting contractions in the colon, thereby promoting laxation<sup>22</sup>. The result of this experiment is at contrast with reports of Shareef *et al*<sup>27</sup>, who reported the antiarrheal and antispasmodic activities of *Operculina turpethum* root. Our report is in agreement with the report of Ajanal *et al*<sup>8</sup>, who reported that excess intake of paste of *Operculina turpethum* along with *Ichhabhedi ras* (herbal formulation) causes drastic purgation. The variation in our reports could be due to differences in the vegetative part of the plant used and climatic condition<sup>30, 31</sup>. The result of this study suggests that *Operculina turpethum* leaves could be administered before surgical, radiological, and endoscopic procedures where an empty colon is desirable<sup>22</sup>. In conclusion *Operculina turpethum* extract showed a potent laxative activity and confirms its use for this purpose in traditional medicine.

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#### CONFLICT OF INTEREST

None declared

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