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## ANTHELMINTHIC AND ANTIBACTERIAL ACTIVITY OF METHANOLIC EXTRACT OF *CUCURBITA MAXIMA* LEAVES

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### ABSTRACT

Helminthic infections are one of the serious diseases affecting humans and animals. The aim of this study to evaluate anthelmintic and antibacterial efficiency of methanolic extract of *Cucurbita maxima* (pumpkin leaves) against Indian adult earthworm *Pheretima posthuma*. Pumpkin leaf extraction done by Accelerated solvent extraction and screened for the presence of phytochemicals both qualitatively and by GC-MS analysis. These procedure methods revealed the presence of flavanoids, terpenoids, saponins, tannins, phenols, carbohydrates, glucosides and sterols. Anthelmintic activity results were expressed regards to paralysis and death. Three worms were released into petridishes containing solutions of different concentrations of extracts (20, 40, 60mg/ml), saline was taken as control and albendazole as standard. Antibacterial activity done by agar well diffusion method using clinical specimens. Antioxidant activity of leaf extract evaluated by DPPH free radical scavenging assay.

### KEYWORDS

*Cucurbita maxima*, *Pheretima posthuma*, Accelerated solvent extraction, Phytochemicals, Anthelmintic, Antibacterial and Antioxidant.

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### INTRODUCTON

Helminthic infections are one of the serious infections to mankind and animals. Helminthic infections are caused by different gastrointestinal parasites. Humans and animals get infected through contaminated food and water.

In humans, helminthic infections cause malnutrition, weight loss, loss of appetite etc. Control of helminthic infections by different drugs are carried out worldwide. But some parasites are resistant to these drugs and also have some side effects. This leads to use of the herbal plants for medicinal purposes (Barnabas B.B *et al*, 2014)<sup>1</sup>.

There are various parasites which include tapeworms, fluke worm, round worm, pinworms etc. Pumpkin is a vegetable of the genus cucurbita and the family cucurbitaceae. Commonly knowing genera are *Cucurbita pepo*, *Cucurbita maxima*, *Cucurbita moschata*, *Cucurbita mixta* etc. Pumpkin is widely grown for food. Its seed leaf and flush are used for different purposes. Pumpkin leaves are rich in phenolics, flavanoids and B carotene (Ko *et al*, 2016)<sup>2</sup>. It is one of the ritual food used by the South Indian, especially in Kerala during karkidaka masa charya.

Pumpkin plant contains calcium, iron, phosphorus, beta carotene, Thiamine, Riboflavin, panthonic acid, vitamin A vitamin C and other bioactive ingredients and these ingredients helpful for the formation of RBC (Yongabi K.A *et al*, 2014<sup>3</sup> and Sengupta R *et al*, 2013<sup>4</sup>). *Cucurbita maxima* has important anthelmintic activity and (Masanta *et al*, 2019)<sup>5</sup>. Pumpkin powders is rich in phenolics and antioxidant activities (Bochnak and Swieca 2020)<sup>6</sup>. Antioxidants play an important role in reduce free radical oxidative stress from body. Leaves *Cucurbita maxima* showed scavenging activity on superoxide radicals at different concentrations of extract (B. Yenda *et al*, 2015)<sup>7</sup>.

Pumpkin is rich source antioxidants help to maintain our health (Peiretti *et al*, 2017)<sup>8</sup>. Antioxidant- components help to reduce the risk of cardiovascular disease, cancer, diabetes mellitus, blood glucose levels, neuro degenerative diseases, osteoporosis etc. (Aune *et al*, 2018<sup>9</sup> Hamer and Chida 2007<sup>10</sup>, Li *et al*, 2012<sup>11</sup>, Zhou *et al*, 2016<sup>12</sup>). *Cucurbita maxima* leaf extract has antimicrobial activity against human pathogens such as *staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *E coli* Also leaves of *Cucurbita maxima* contains minerals and proximate s necessary for growth and maintenance of body (Okon *et al*, 2014)<sup>13</sup>.

Hence the present study was to evaluate the efficiency of Anthelmintic Activity and antibacterial of pumpkin leaf extract as a control of Anthelmintic and antibacterial infection against Indian earthworm, *Pheretema posthuma* as a model.

## MATERIAL AND METHODS

### Collection of plant material

The leaves of cucurbita maxima (pumpkin) were collected from Vadakkenchery, Palakkad district, Kerala. The leaves were washed thoroughly with tap water to remove dirt's and dried for 3-5 days under shade at room temperature.

### Preparation of extracts

The dried leaves were ground using motor and pestle to obtain fine powder. Ground sample loaded into extraction cell of accelerated solvent extractor. Methanol used as Solvent. The obtained extract kept stored at 4°C in refrigerator.

$$\% \text{ of yield of extraction} = \frac{\text{Weight of dry extract} \times 100}{\text{Weight of dried powder}}$$

### Phytochemical screening

Phytochemical screening of plant extracts carried out for the presence of alkaloids, flavanoids, terpenoids, phenol, tannins, saponin, carbohydrates, glycosides according to methods described by Harbone.

### Anthelmintic Activity

Indian Adult earthworms were used for the study due to anatomical and physiological resemblance with intestinal helminths. Adult *Pheretima posthuma* with approximately equal size was used in this study. The earthworms were collected from Kerala Agricultural University, Mannuthy and cleaned with distilled water. Prior to experiment, the earthworm was acclimated in saline solution for 30 minutes. The experiment divided into 5 groups. Each group consisted of 3 earthworms. The earthworm was placed into petri dish containing 20ml of solution. Group I negative control was normal saline. Group II served as standard albendazol (20mg/ml) was used as positive control. Group III, IV, V were treated by the plant extract at the concentration of 20, 40 and 60mg/ml, respectively. Paralysis and death of individual earthworm were observed for 3 hours and recorded the time in minutes. Paralysis was recorded if there is no movement of the worms except when shaken vigorously. Death was considered when there is no movement of worms followed by fading of body colour (Sutnga *et al*, 2020)<sup>14</sup>.

### Antibacterial Activity

Microorganisms used for the study collected from nearby hospital. Organism used includes *Escherichia coli*, *Staphylococcus aureus*, *Bacillus sp*, *Klebsiella sp*. Antibacterial activity done using agar well diffusion method.

### GC MS analysis

GC MS analysis was done using system Shimadzu GC-MS, Model Number: QP2010S

Column ELITE-5MS, 30 meter length, 0.25mm ID 0.25µm thicknesses.

Column Oven Temp	80.0°C
Injection Temp	260.00°C
Injection Mode	Split
Flow Control Mode	Linear Velocity
Pressure	65.0kPa
Total Flow	23.9mL/min
Column Flow	1.00mL/min.

### ANTIOXIDANT ACTIVITY

#### DPPH radical scavenging assay

The radical scavenging activity of different extracts was determined by using DPPH assay according to (Chang *et al*, 2001)<sup>15</sup>. The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517nm. Ascorbic acid (10mg/ml DMSO – Dimethyl Sulfonate) was used as reference.

#### Statistical analysis

Anthelmintic activities were expressed as Mean ± SEM (standard error of mean).

### RESULTS AND DISCUSSION

#### Extraction yield

#### Phytochemical screening

Methanolic extract of *C maxima* leaf had the presence of bioactive components (Table No.3).

#### Anthelmintic activity

In this study anthelmintic activity of methanolic extract of pumpkin leaves was carried out using adult indian earthworm revealed that pumpkin leaves extract has important role in anthelmintic activity based on the paralysis and death time.

### Antibacterial activity

Methanolic extract of pumpkin leaves has high activity against *Staphylococcus aureus* and *Bacillus sp* and low activity against *E coli* and *Klebsiella sp*.

### GC-MS Analysis

GC-MS analysis revealed 12 components in methanolic extract of pumpkin leaf (Figure No.3 and Table No.5). In which major component was dl- $\alpha$ -Tocopherol with highest area percentage (41.93). It is one of the form of Vitamin E, fat soluble antioxidant (Tucker *et al*, 2005)<sup>16</sup>.

### Discussion

In this study qualitative phytochemical screening showed the presence of flavonoids, terpenoids, saponins, phenols, tannins, carbohydrates, glycosides and phenols. Flavonoids possess a number of medicinal benefits, including anticancer, antioxidant, anti-inflammatory, and antiviral properties. They also have neuroprotective and cardio-protective effects (Asadh Ullah *et al*, 2020)<sup>17</sup>. Terpenoids hasbiological activities including anticancer, antimicrobial, anti-inflammatory, antioxidant, and antiallergic, it also exhibit antimicrobial and food preservative properties. Saponins founds in medicinal plants have important properties to protect plants from herbivores, antimicrobial and other pharmaceutical properties like anticancerous, antiviral activities (Mugford *et al*, 2012)<sup>18</sup>.

Anthelmintic activity of *C maxima* leaves done on indian adult worm (*P posthuma*). *C maximahas* important role in anthelmintic activity was proved by the experiment based on the paralysis and death of worms. It was cleared that the paralysis and death of worms in the petridish contained the worms with Albendazole and petridish contained the worms with leaf extract were almost similar. Experiment also showed the highest concentraion 60mg/ml has high activity compared to other concentrations, 20mg/ml and 40mg/ml.

GC-MS analysis displayed presence of 12 componens. Major component was found to be the dl- $\alpha$ - tocopherol, vitamin E rich in antioxidant components. GC-MS analysis revealed the presence of lipids, triterpenoids, alkanes, phytols etc, each

components have important pharmaceutical applications.

In this study antioxidant activity by DPPH radical scavenging assay methanolic extract of pumpkin leaves showed relevant antioxidant activity with IC<sub>50</sub> value 89.295µg/ml compared to the standard, ascorbic acid.

**Table No.1: Preparation of extracts**

S.No	Parameter	Accelerated Solvent extraction
1	Solvent	Methanol
2	Temperature	60°C
3	Pressure	1500 psi
4	Heating	4 minutes
5	Static time	5 minutes

**Table No.2: Extraction yield**

S.No	Parameter	Accelerated Solvent Extraction at 60°C
1	Time	20 min
2	Solvent consumption	150ml
3	Sample weight	16g
4	Extract weight	5g
5	Percent yield	31.25

**Table No.3: Qualitative phytochemical screening**

S.No	Plant contents	Methanolic extract of <i>C maxima</i> leaves
1	Alkaloids	-
2	Flavanoids	+
3	Terpanoids	+
4	Saponin	+
5	Tannin	+
6	Phenols	+
7	Carbohydrates	+
8	Glycosides	+
9	Sterols	+

**Table No.4: Anthelmintic activity**

S.No	Group	Concentration (mg/ml)	Paralysing time (Minutes)	Death Time (Minutes)
1	Normal Saline (control)	-	-	-
2	Standard (Albendazole)	20	31 ± 0.58	130.17 ± 0.93
3	Methanolic Extract	20	65.17 ± 0.73	162.17 ± 0.44
4	Methanolic Extract	40	45.27 ± 0.37	143.50 ± 0.76
5	Methanolic Extract	60	34.97 ± 0.55	135.00 ± 0.58

Results are expressed as Mean ± SEM (standard error of mean), n=3

**Table No.5: Compounds in methanolic extract of pumpkin leaf in GC-MS**

Peak	R.Time	Area%	Height %	Name	Molecular Formula
1	26.344	16.45	20.09	2, 6, 10-trimethyl, 14-Ethylene-14-Pentadecne	C20H38
2	26.852	2.55	3.53	Phytol, Acetate	C20H40
3	27.224	4.62	5.75	3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol	C20H40O
4	31.686	17.75	15.99	2-Hexadecen-1-OL, 3, 7, 11, 15-Tetramethyl-, [R-[R*, R*-(E)]]-	C20H40O
5	34.647	1.2	1.57	3-Cyclopentylpropionic acid, 2-dimethylamino ethyl ester	C12H23NO2
6	37.614	1.61	1.78	2-Propenoic acid, 2-Methyl-, 2-(Dimethylamino) Ethyl Ester	C8H15NO2
7	38.617	1.39	1.64	Octadecanal	C18H38O
8	41.011	1.31	1.8	Docosane	C22H46
9	42.908	5.35	7.58	Squalene	C30H50
10	43.790	1.17	1.63	Eicosane, 7-Hexyl-	C26H54
11	46.378	4.68	4.33	.gamma.-Tocopherol	C28H48O2
12	17.857	41.93	34.31	dl-alpha.-Tocopherol	C29H50O2

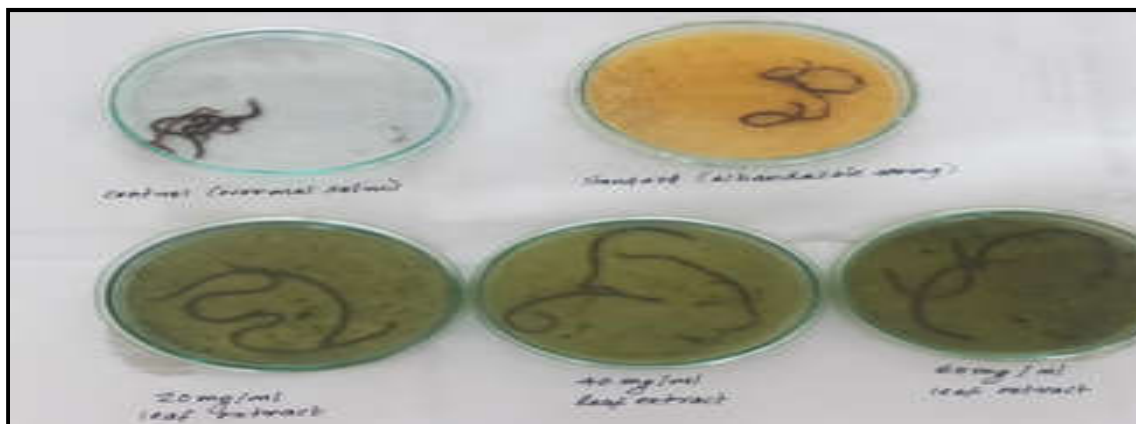
**Table No.6: DPPH radical scavenging assay**

S.No	Concentrations (µg/mL)	Absorbance	Percentage of inhibition
1	Control	0.3584	0.00
<b>Standard: Methanol</b>			
3	12.5	0.3264	8.92857
4	25	0.2755	23.1306
5	50	0.2401	33.0078
6	100	0.1656	53.7946
7	200	0.0966	73.0469

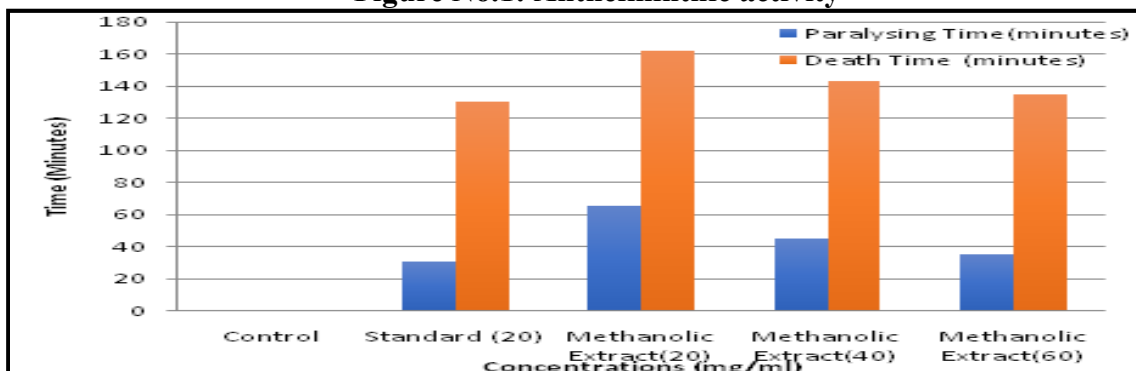
IC 50 value- methanol- 89.2952µg/ml

**Table No.7: Standard DPPH Radical scavenging activity**

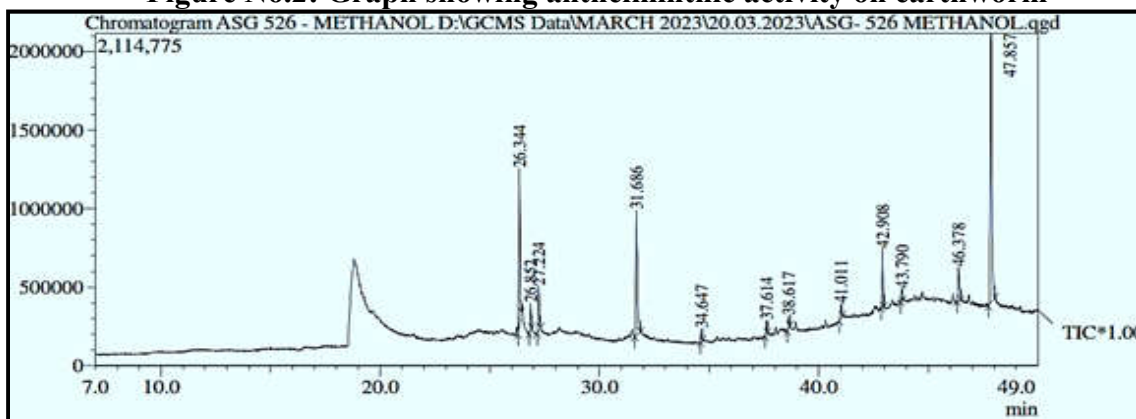
S.No	Standard: Ascorbic Acid		
1	12.5	0.2654	26.8266
2	25	0.1745	51.8886
3	50	0.1236	65.9222
4	100	0.0654	81.9686
5	200	0.0216	94.0447



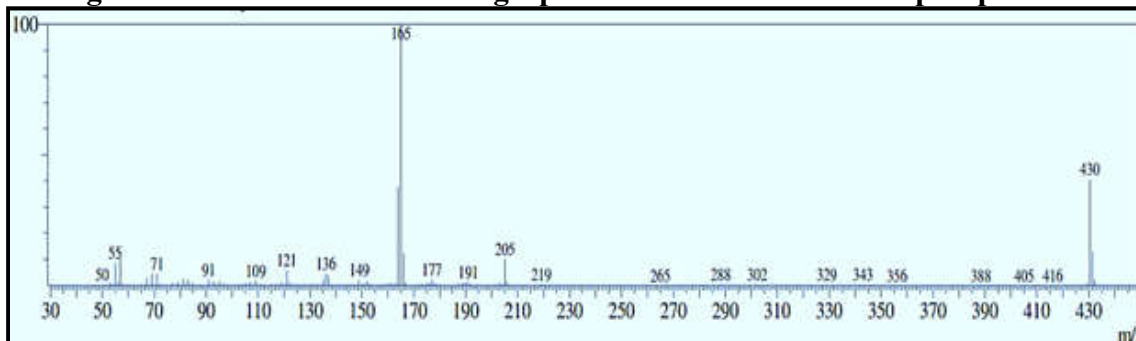
**Figure No.1: Anthelmintic activity**



**Figure No.2: Graph showing anthelmintic activity on earthworm**



**Figure No.3: GC-MS Chromatogram of methanolic extract of pumpkin leaf**



**Figure No.4: GC-MS Chromatogram of compound dl-.alpha.-Tocopherol**

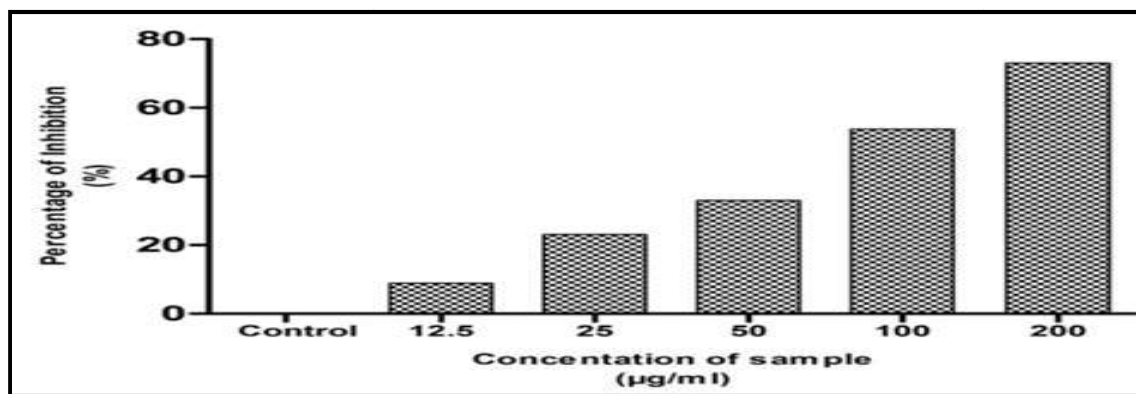


Figure No.5: Graphical representation depicting the DPPH activity of Methanol- along Y axis Percentage inhibition, along X axis varied concentration of Methanol

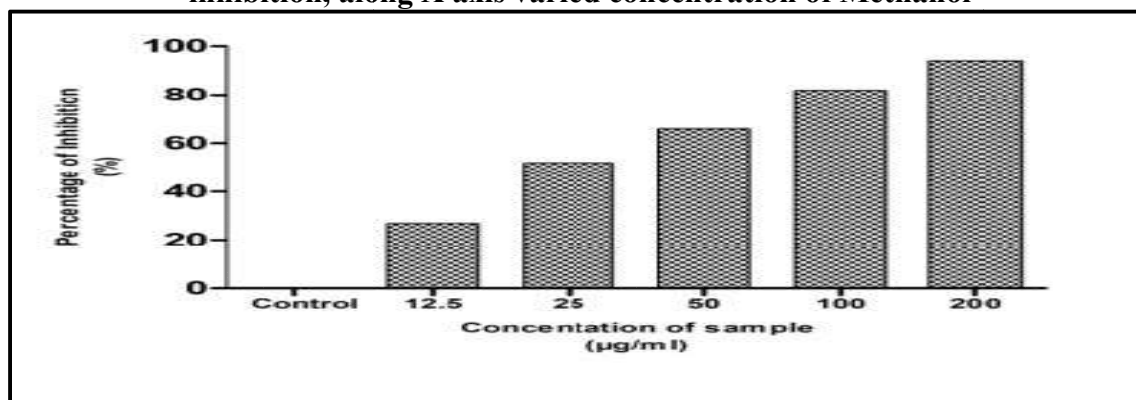


Figure No.6: Graphical representation depicting the DPPH activity of ascorbic acid-along Y axis Percentage inhibition, along X axis varied concentration of ascorbic acid

## CONCLUSION

Present study concluded that various phytochemical constituents present in the methanolic extract of *Cucurbita maxima*. It is evident that pumpkin leaf has significant anthelmintic and antibacterial activity. GC-MS study analysis revealed the presence of components that will reduce the risk of many diseases. Based on the study pumpkin leaf has important activity against helminths and can be formulate candies and syrups in future as a control of helminthic infection.

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## CONFLICT OF INTEREST

We declare that we have no conflict of Interest.

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