

## **Allelopathic Properties of *Ipomoea Hildebrandtii* Vatke**

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### **Authors' contributions**

This work was carried out in collaboration among all authors. Authors NG did the laboratory analysis, author PK procured the reagents, authors MG and GN took part in guidance in the project and literature review. All authors read and approved the final manuscript.

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### **ABSTRACT**

The use of synthetic pesticides to alleviate the notorious weeds in farms has caused environmental pollution, consumption of chemicals absorbed by plants and resistance posed by the weeds putting agricultural development and ecological systems under threat. The main reason for study of allelopathic effect of the plant include; its application of observed allelopathic effects to agricultural production, reduction of inputs to chemical pesticides uses, resultant environmental pollution and for provision of fruitful method for sustainable development of agriculture and ecological systems. This study made the use of allelochemicals produced by the invasive *Ipomoea hildebrandtii* to provide an effective method of controlling weeds through biological control method which is environmentally friendly as compared to the conventional chemical method. Extraction was done and concentrated by rotary evaporator, pH of the soil under which the plant grew was determined. The allelopathic test was done at different concentrations in petri dishes on *Phaseolus vulgaris* and the characterization of the plant compounds done by Ultra Violet coupled to visible region, Fourier Transform Infra-Red, and Gas Chromatograph -Mass Spectroscopy. The leave extract was more effective in allelopathic effect on the plant than the stem extract, that means the inhibiting compounds are more concentrated in the leaves than the stem.

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## 1. INTRODUCTION

Allelopathy is a biological phenomenon by which an organism produces one or more secondary metabolites that influence the growth and development of biological systems with positive and negative effects on the target organism and the community [1].

According to studies done the allelochemicals are a subset of secondary metabolites which are not required for the metabolism of an allelopathic organism. Concerning the work done by allelochemicals with negative allelopathic effects are an important part of plant defence against herbivory adaptation evolved by plants to improve their survival [2].

Allelopathic effects are an important factor in determining species distribution and abundance within plant communities and are important in the success of many invasive plants like *Ipomoea hildebrandtii*. Allelopathy as a science can be used in the control of diseases and weeds which compete against important plants, providing raw material for phytotherapeutic industry allelopathic organism can find application in areas of agriculture, by observing the allelopathic effects to agricultural production [3] reduction of inputs to chemical pesticides and consequent environmental pollution and provision of effective methods for sustainable development of agricultural production and ecological systems. A focus on the effects of weeds on crops, crops on weed, and crops to crops has opened up the possibility of using allelochemicals as growth regulators and natural herbicides to promote sustainable agriculture.

*Ipomoea* weed is a creeping annual herb that is widely spread in semi-arid parts of southern Kenya especially kajiado county it invades and spreads at a higher rate mostly after the inception of rainy period. The weed is notorious and causes problems in natural pastures and to some extent to well establish pastures for kajiado county residents. According to he described *Ipomoea* weed as the most objectional fodder species for grazing of livestock [4].

The invasive *Ipomoea* produces a secondary metabolite that injures neighbouring plants and soil community through direct toxicity. The seed germination tests and initial radicle growth in the presence of medicinal plant extracts are of great

scientific interest since lots of allelochemicals are utilized in traditional medicine for the treatment of several diseases. The allelopathic interaction can be a source of potential natural phytotoxic compounds that is of low toxicity to non-target control organism [5].

Allelopathic weeds such as *Ipomoea hildebrandtii* and *Eucalyptus globulus* Labill can constructively be used to control annoying weeds near crops by planting a variety with allelopathic qualities, [6] on the other hand, the use of allelopathic compounds before or after the application of synthetic herbicides can increase the total effect of both materials hence by doing so, it will reduce application rates of synthetic herbicides [7].

The use of *Ipomoea hildebrandtii* for weed control can be effective as it is easy to cultivate and easy to maintain. There was a significant inhibition of development of radicle and plumule of treated *Cassia fistula* and *Amaranthus spinosus* seeds treated with *Ipomoea hildebrandtii* extracts showed inhibition to root and shoot development as compared to control seeds treated with water. According to the work done, [8] there was a significant reduction in root and shoot length on test seedlings due to allelopathic effects of *Ipomoea hildebrandtii* extracts. In other findings, a study conducted in Pakistan where 239 plant species of medicinal origin were studied for their allelopathic effects using the sandwich method and they noted a decrease in germination percentage on the seeds tested and they recommended that allelopathic studies need to be conducted in a standardised manner to study plant species that have allelopathic activity.

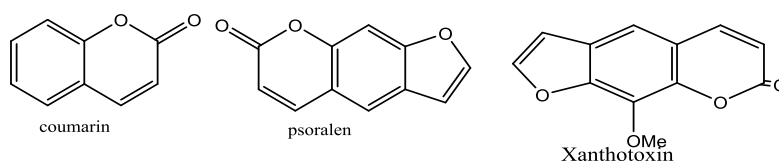
The ethyl acetate fraction of the *Ipomoea hildebrandtii* extract may contain a large part of phenolic compounds that are responsible for inhibiting germination power since most of those compounds act at the cellular level and on the plasma membrane interfering a range of cellular processes with the release of reactive oxygen species [9]. As stated by research findings, phenolic present in the *Ipomoea hildebrandtii* leaves are capable of hindering the action of gibberellins by either interacting with the molecules or by blocking processes mediated by them as a reduction of synthesis of hydrolytic enzymes like amylases and acid phosphatase in the endosperm of the seed thus hindering germination process.

In line with previous studies done on allelopathic effects of *Ipomoea hildebrandtii* species showed that terpenoids and phenolic compounds were responsible for the inhibitory property of the species under *Ipomoea hildebrandtii*. According to the study conducted, HPTLC analysis of *Ipomoea carnea* showed the presence of tannins, terpenoids, and flavonoids which are responsible for allelopathic inhibition of certain weeds such as *Amaranthus spinous* and *Cassia fistula* [10]. The indicated compounds according to literature include coumarins which are found in more than 70 plant families, they are said to lower the oxygen uptake by the plants and cause changes in the structure of the mitochondria [11]. The compound can inhibit growth in some parts of a plant or the whole plant. coumarin structures are as shown in the following figures [12].

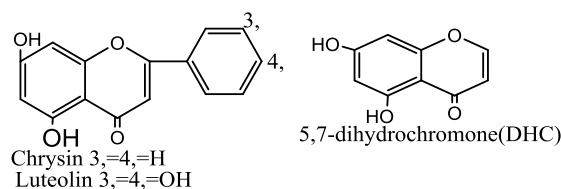
Flavonoids are another diverse compound that can be found both in lower and in a higher plant. Flavone is a subgroup of the compound and contains ketone oxygen, it is known to inhibit the growth of other plants as well as fungi. The structures are as shown.

Alkaloids are known to have wide biological functions; they regulate the growth of seeds. The common alkaloids are gramine and hordenine. They are known to inhibit seed germination of other plants and hence eliminating growth competition. The structures of the named alkaloids are as shown [4].

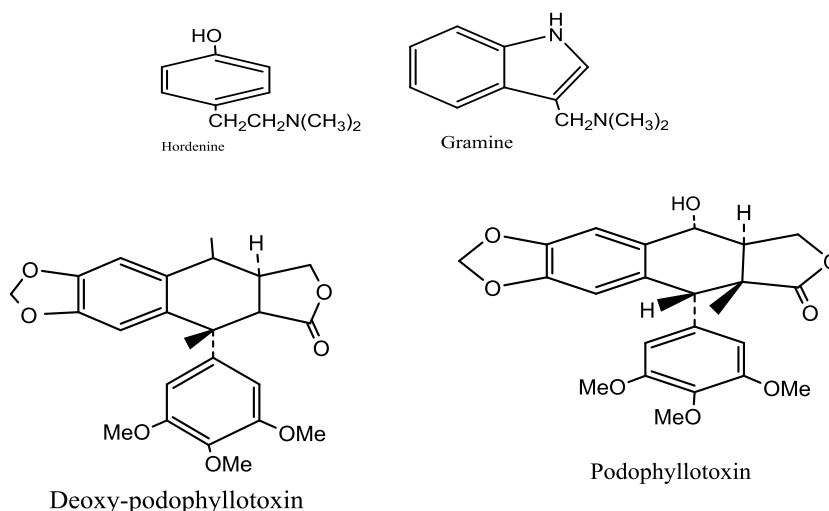
Lignans are the other compounds found in large quantities and are known to inhibit the growth of the other plants. They include mimosine, nebularine, steroidal glycosides, podophyllotoxins. The structures are as drawn.



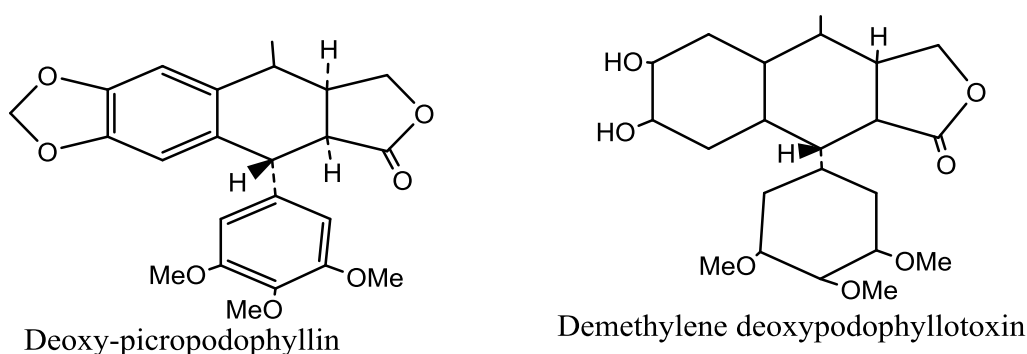
**Fig. 1. Coumarins, well known inhibitors of plant growth [13]**



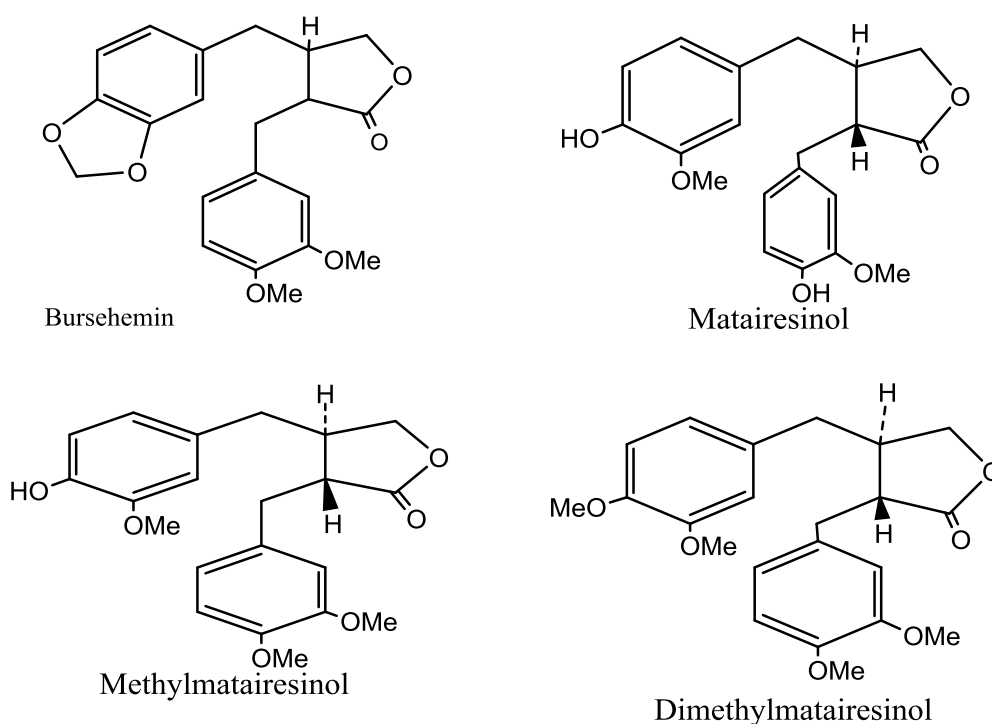
**Fig. 2. flavonoids-plant growth regulators[14]**



**Fig. 3. Lignans (podophyllotoxins)-plant growth regulators [15]**



**Fig. 4. Lignans (podophyllotoxins)-plant growth regulators [15]**



**Fig. 5. Lignans (matairesinol related compounds)-plant growth inhibitors [15]**

## 2. MATERIALS AND METHODS

Plant stems and leaves were collected from kitengela, Kajiado County in Kenya, washed, dried at room temperature for a week, and ground ready for extraction.

### 2.1 Solvent Extraction

10 g of each plant parts were put into different conical flasks, 250 ml of hexane was introduced which later followed with ethylacetate and methanol extraction process. The conical flasks having each different plant was subjected to ultra-sonification for 30minutes in ultrasonic bath. Ultrasonicated sample was incubated in an oval-

rotating incubator fixed at 180rpm for 25hrs at 25°C [4]. Resulted suspension was then filtered by Whatman's filter paper to obtain different plant parts extract. The process was repeated with the same samples in ethylacetate then methanol to capture compounds with different polarities. The process of extraction was also done for *Eucalyptus globulus* for germination inhibition comparison.

The synthesis of Iron complex was done by adding 0.01 M Ferrous Chloride to ipomea and eucalyptus extracts in the ratio of 2:5 proportion in a clean sterilized flask. The reacting mixture were placed in a magnetic hot stirrer at 50-60°C for an hour. The change of colour from pale

green to brown signified oxidation of the ions. The solution was put in a centrifuge with 350 rpm for ten minutes and the supernatant was put away. The resulting pellet was then washed with distilled water and kept for the bioassays.

## 2.2 Growth Inhibition

The experiment was done using extracts in Petri dishes with serial dilutions from 0, 0.1, 0.2, 0.5, 1, 2, 3 and 5 %v/v respectively which were prepared by soaking dried ground residue in distilled water at room temperature for 24hrs in 250 ml Erlenmeyer flasks. Extracts were then filtered through Whatman filter paper. *Phaseolus vulgaris* and corn seeds were disinfected for 3 minutes in 1% sodium hypochlorite, then rinsed in distilled water. Ten seeds were placed in separate Petri dishes (10 cm) with filter paper 10ml of each extract added. Petri plates were kept in a dark incubator at 24°C and 100% relative humidity. After 72 hours, coleoptile and radicle lengths were calculated and the number of secondary roots recorded [11].

Dilutions of 0, 0.1, 0.2, 0.50, 1.0, 2.0, 3.0, and 5.0 % of the residue were incorporated in the silica sand where *Phaseolus vulgaris* and corn were planted, Hoagland's solution was supplied by surface watering. Height and fresh weights of the shoot in corn and *Datura stramonium* and the length of the first internode, heights and fresh weights of shoot and roots in corn recorded after 28 days after planting.

## 2.3 UV-Visible Spectra Analysis

The measurement of the UV-Visible absorption spectra and their complexes was done on a Shimadzu UV-Vis 530A spectrophotometer in the range of 200-800nm [7]. The peaks of the absorption of these complexes and those considered to be pure extracts were compared and peak absorption variations were recorded.

## 2.4 FT-IR Analysis

FT-IR spectrum at a resolution of 4cm<sup>-1</sup> and in the range 4000 to 400cm<sup>-1</sup> using Perkin-Elmer spectrometer was used to sense the complexities in the functional groups. The sample was mixed with potassium bromide. Preparation of thin sample discs done by pressing the disc preparing machine and placed in Fourier Transform Infra-Red (FTIR) for the later complexity analysis.

## 3. RESULTS AND DISCUSSION

The pH of the soils around the plant was acidic due to the decayed fallen leaves of the ipomea plant, 3 meters away the PH was slightly higher because the effect of the ipomea did not reach here, and at 6 meters where there was grass growth the PH was slightly low as recorded in the table.

The extraction was done from the three solvents, this is attributed to the fact that compounds in the plants have different polarity ranges.

The inhibition of ipomea leaves extract on *vulgaris* was done and recorded as shown above. At 5% extract concentration, there was no growth at all and the germination rate of the seeds increased with decrease in extract concentration, this explains that the plant leaves have allelochemicals that inhibits growth of the other plants. The control (water/no extract) indicated the highest germination.

Allelopathic effect was also done for the stem extracts, concentrations ranged from 0.1% to 5.0%. From the results, there was no growth at 5% concentration and the germination rate increased with the decrease in extract concentration. It is also noted that the allelopathic effect is higher in the leaves as compared to the stem.

**Table 1. pH of soil samples (*Ipomoea hildebrandtii*)**

Top soil on the plant growth			Top soil 3metres from the plant			Top soil 6metres from the plant		
6.6	6.58	6.72	7.25	7.10	7.15	6.26	6.10	6.18
Av=6.65±0.07			Av=7.17±0.076			Av=6.18±0.080		
30 cm depth on the plant			30cm depth, 3mtr from the plant			30 cm depth, 6mtr from the plant		
7.13	7.20	7.17	7.40	7.38	7.32	6.52	6.48	6.43
Av=7.17±0.035			Av=7.29±0.042			Av=6.48±0.045		

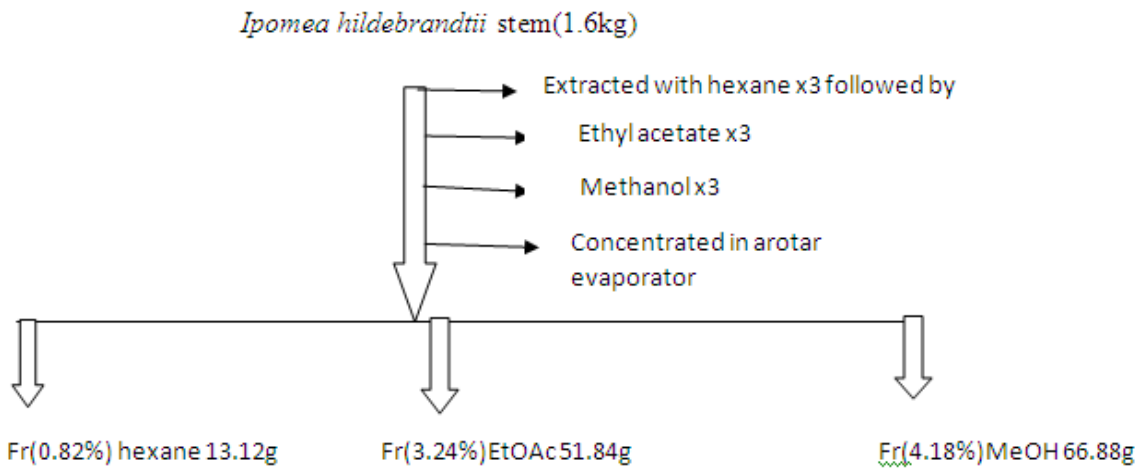


Fig. 6. Solvent extraction of *Ipomea hildebrandtii* stem



Fig. 7. Allelopathy of *Ipomea hildebrandtii* leaves extract at different Concentrations in *Phaseolus vulgaris* growth for 72hrs

Table 2. The inhibition of ipomea leaves extract

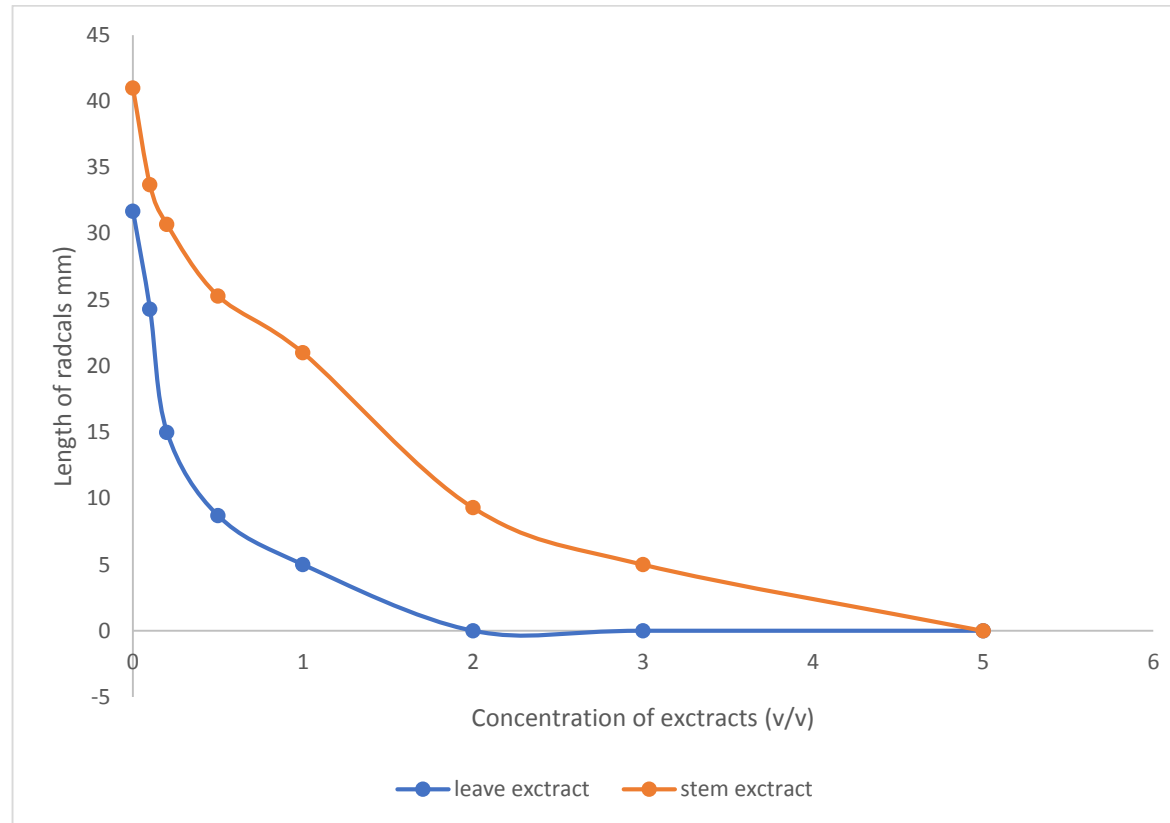
Conc in % v/v	Control (0.0%)	0.1%	0.2%	0.5%	1.0%	2.0%	5.0%
Radicle length in mm	40 28 27	28 29 16	18 15 12	12 8 6	8 5 2	0 0 0	0 0 0
Av±SD	31.7±7.23	24.3±7.23	15±3.00	8.7±3.06	5.0±3.00	0±0.00	0±0.00



Fig. 8. Allelopathy of *Ipomea hildebrandtii* stem extract at different concentrations in *Phaseolus vulgaris* growth for 72hrs

**Table 3. Allelopathic effect for the stem extracts**

Conc in v/v	Control (0.0%)			0.1%			0.2%			0.5%			1.0%			2.0%			3.0%			5.0%		
Radicle length in mm	42	43	38	37	28	36	35	25	32	20	32	24	25	20	18	10	12	6	8	5	2	0	0	0
Av±SD	41±2.65			33.7±4.93			30.7±5.13			25.3±6.11			21.0±3.61			9.3±3.06			5.0±3.0			0.0±0.0		



**Fig. 9. Effect of various concentration of leaves and stem extract on the growth**

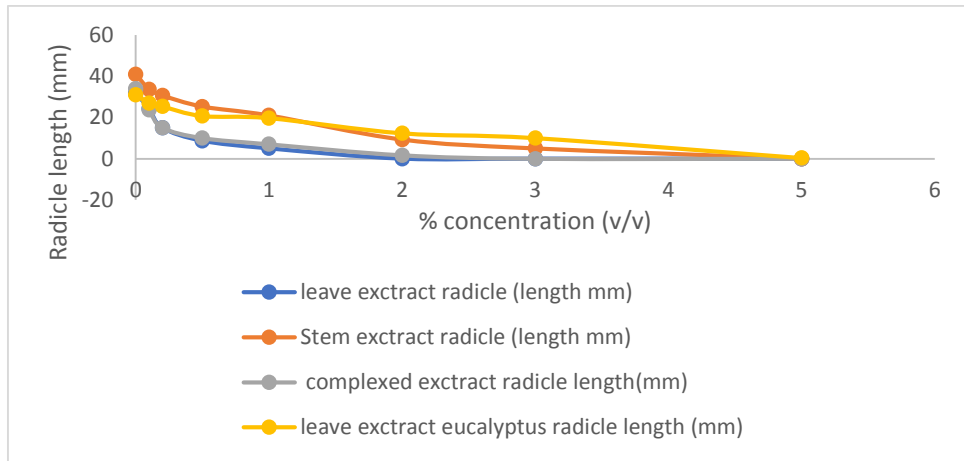


Fig. 10. Effect of various concentration of leaves and stem extract radicle on the length

### FT-IR SPECTRA OF LEAF EXTRACT

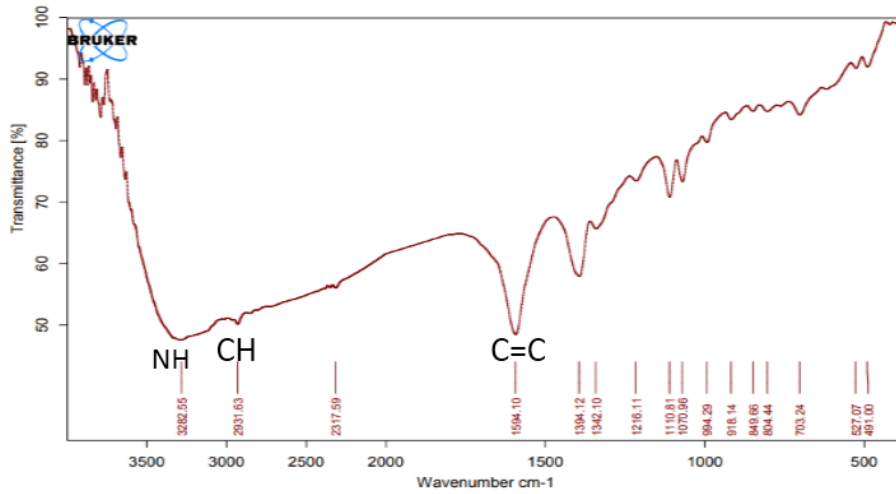


Fig. 11. FT-IR spectra of leaf extract

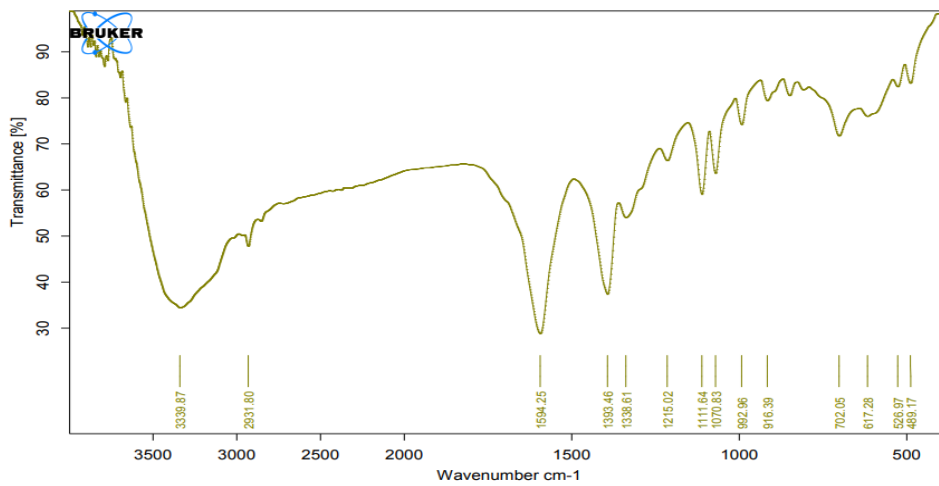


Fig. 12. FT-IR Spectra of leaves extract complexed with iron ii



## FT-IR SPECTRA OF STEM EXTRACT

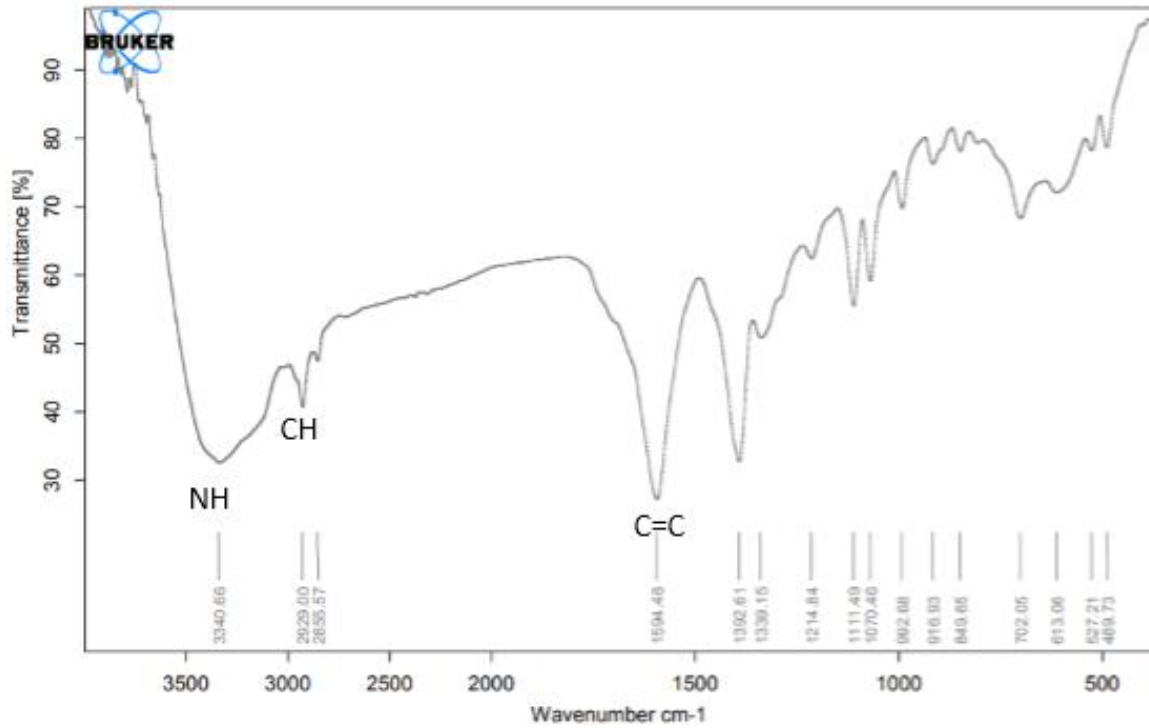


Fig. 13. FT-IR spectra of stem extract

## FT-IR SPECTRA OF STEM EXTRACT COMPLEXED WITH IRON II

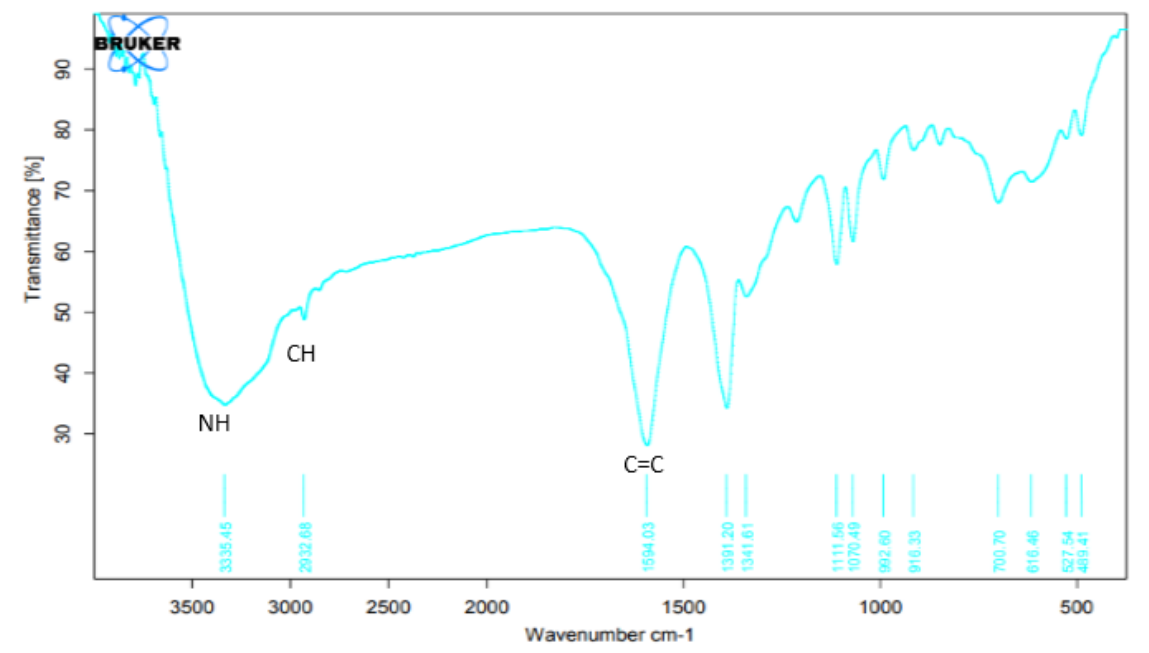


Fig. 14. FT-IR spectra of stem extract complexed with iron ii

## UV-VIS SPECTRA OF PURE AND COMPLEXED EXTRACT WITH Fe(ii)

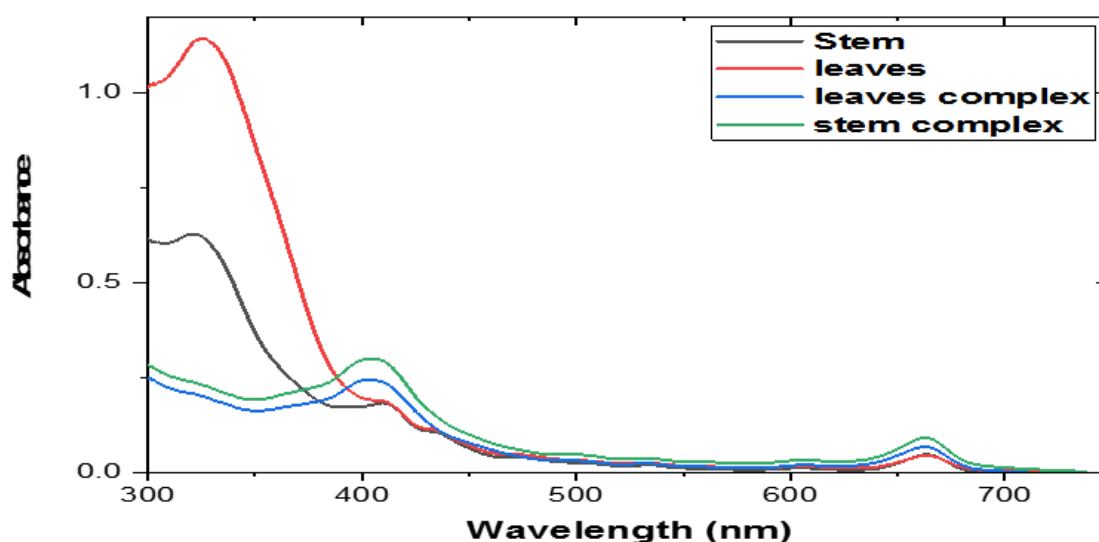


Fig. 15. UV-VIS spectra of pure and complexed extract with Fe(ii)

Graphical representation of the effect of various concentration of leaves and stem extract on the growth of *Phaseolus vulgaris* radical length (mm) after 72 hours incubation in the dark at 24°C. The leaf extract was more effective than the stem extract that means the inhibiting compounds are more concentrated in the leaves than the stem.

Comparison of allelopathy of eucalyptus leaf extract and *Ipomoea hildebrandtii* leaf extract, stem and complex of iron (II) extracts on the growth of *Phaseolus vulgaris*, radical length (mm) after 72 hours incubation in the dark at 24°C. Complexed and leaf extract of *Ipomoea hildebrandtii* were more effective in the inhibition of *Phaseolus vulgaris* radicle growth than the stem extract, and eucalyptus globulus extract.

The FTIR indicated the functional groups that are found in the plant.

The peaks indicated the functional groups that are present in the plant. Some of the bands observed in the complexed spectrum were different in intensity compared to those of the uncomplexed one. N-H, C-H and C=C groups were at a slightly different location from those of uncomplexed extract. The shifting of the peaks for complexed extract is attributed to the formation of iron II nanoparticles by the N-H, C=C groups.

### 3.1 UV-VIS Peaks

The observed absorption wavelength of the complexed extract indicated a shift towards the shorter wavelength at 410 nm and 670 nm. This shifting is attributed to the reduction of conjugation as a result of the reaction between the metal ions and the lone pairs and pi electrons in the compounds present in the extract. For *Ipomoea* leaves, stem and complexed materials there is the enhancement of the peak intensities at approximately 410 nm and 670 nm.

## 4. CONCLUSION

From the experiment, the growth of *Phaseolus vulgaris* was inhibited by the highest concentration of the *Ipomoea* as compared to eucalyptus, this was seen from the rate of the growth observed after the subjection of the inhibitor hence the plant can be applied biologically to inhibit the growth of weeds. The plant was seen to contain the compounds discussed.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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