COMPARATIVE ASSESSMENT OF PHYTOCHEMICAL PROFILES, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF *LANTANA CAMARA* LEAF EXTRACTS

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Lantana camara L. is a widespread invasive weed that native to the tropical regions of Africa, Asia and America. This study investigated its potential benefits by extracting active components from its leaves using various methods including conventional (aqueous extraction) and nonconventional (ultrasonic-assisted ethanolic and enzyme-assisted extraction). The polyphenols and phytohormones profiles of the extracts were characterized by high-performance liquid chromatography. The antiproliferative activity of the extracts was assessed for antibacterial and nematicidal potentials. Notably, the enzyme-assisted extract had better antibacterial activity compared to the other methods. Additionally, the extracts exhibited varying degrees of nematicidal activity, showing significant efficacy against *Meloidogyne* juveniles. Furthermore, the general biological activity of the extracts was evaluated using seed germination bioassay. Interestingly, the ultrasonic-assisted ethanolic and enzyme-assisted extracts significantly stimulated wheat seedling growth. In contrast, the aqueous extract displayed the highest phytochemicals and antioxidant activity compared to the ultrasonic-assisted ethanolic and enzyme-assisted extracts. This suggests that different extraction methods may target different bioactive compounds with diverse biological effects. In conclusion, this study suggests that *L. camara* extracts, particularly those obtained through enzyme-assisted methods, possess promising antibacterial, nematicidal, and antioxidant properties. This may open further research and potential development of natural alternatives for various applications.

Keywords: *Lantana camara*, enzyme-assisted extract, ultrasonic-assisted ethanolic extract, phytochemical analysis, antiproliferative activity, biostimulant, *Meloidogyne*

INTRODUCTION

Plants offer tremendous natural compounds with antimicrobial and antioxidant properties, which have potency in suppressing both plant and human diseases. The first crucial step in harnessing their medicinal and agriculture potential is screening and identification of such compounds from diverse plant species. In agriculture, the application of antimicrobial phytochemicals as part of an integrated disease management strategy would reduce the reliance on synthetic chemicals (Mansoori et al., 2020)

Lantana camara L., a member of the Verbenaceae family characterized by having a total of 37 widely distributed genera including *Lantana*, is an evergreen shrub known by red, white, and wild sage (Barik et al., 2020 and Kato-Noguchi and Kurniadie, 2021). *Lantana* is a well-known genus of this family used as hedges and for decorating gardens, encompassing a total of 150 species. Among these, *L. camara* is the most prevalent (El-Din et al., 2022).

L. camara is a tropical plant native to Central and Southern America (Nel et al., 2004). It is an extremely invasive weed, which has invaded other tropical and subtropical regions. This spread likely happened throughout the 19th and early 20th century (Mack et al., 2000). It has invaded much of Sub-Saharan Africa and nearly 50 other countries globally, forming dense clusters that threaten natural communities (Barik et al., 2020 and Agaldo, 2020).

L. camara has multiple purposes, mainly as herbal medicine and as firewood (Saraf et al., 2011). Parts of *L. camara* such as leaf, flower, stem and roots are known for its abundance of various phytochemicals, including flavonoids, alkaloids, tannins, and terpenoids. These compounds contribute to its potential health benefits. Traditionally, *Lantana* has been used to treat a wide range of ailments like cancers, tumors, skin conditions, fevers, asthma, and rheumatism (Abu-Shanab et al., 2006; Saxena et al., 2012 and Battase and Attarde, 2021). Recent studies by El-Din et al. (2022) found that leaf extract of *L. camara* may possess anti-inflammatory, antioxidant, and even anticancer properties.

Root-knot nematode (*Meloidogyne* spp.) is the one of most damaging groups of plants. It is an obligatory root parasite of more than 200 plant species including vegetable, horticulture and woody plants (Hussey, 1985). The root-knot nematode is considered as the major problem for many agriculture crops (Keshari, 2004). Therefore, the management of root-knot nematodes is very important to enhance plant productivity. Since chemical pesticides cause hazard to biodiversity, the use of botanical pesticides can be better option to control root-knot nematodes.

The application of plant extracts is one of the outstanding alternative control measures against the nematode (Balah and AbdelRazek, 2020). Plant extracts have been shown to take advantage of the benefits of phytochemicals in plants, an appropriate extraction method should be chosen. There are a wide range of technologies with different extraction methods that have been used to extract phytochemicals, including conventional and nonconventional methods (Azwanida, 2015 and De Silva et al., 2017). Conventional methods are typically simple and inexpensive, but they can be time-consuming and require large amounts of solvent. In Contrast, nonconventional methods of extraction "green techniques" are often more efficient and less time-consuming than conventional methods, but they can be more expensive and require more specialized equipment (Sik et al., 2020). Biological enzyme-assisted extraction is a promising modern approach for extraction. Biological extraction depends on the occurrence of selective enzymes that can break down plant cell wall polysaccharides, increasing the yield of phytochemicals from intracellular compartments (Zhang et al., 2017). Thus, biological enzyme- assisted extraction possesses the advantages of being environmentally friendly, highly efficient and offers a simplified extraction process (Li et al., 2012). The enzyme- assisted extraction method has shown to improve the extraction yields and to enhance the recovery of bioactive compounds in Sargassum (Puspita et al., 2017). Therefore, using *L. camara* phytochemicals is an important issue considering the significance of these substances as biological agents that benefit both the agriculture and human health sector.

The present study aims to compare the efficacy of three different extraction methods, namely aqueous, ultrasonic-assisted ethanolic, and enzyme-assisted extraction, for isolating phytochemicals from *L. camara* leaves. These extracts will be evaluated for biological activities. By comparing the different extraction techniques, this study seeks to determine the most effective method for obtaining a broad spectrum of phytochemicals with desirable biological effects.

MATERIALS AND METHODS

1. Preparation of Plant Material

L. camara fresh leaves (Fig. 1) were collected and thoroughly washed, dried, and separated into three equal portions, each weighing approximately 200 g. After mincing the leaves, the first portion was designated for biological extraction, while the remaining two portions were dedicated to aqueous and ethanolic extraction.

Fig. (1). A photo of *Lantana camara.*

1.1. Aqueous extraction

Aqueous extraction was performed according to Muhamad and Mat (2019) with some modification. Two hundred grams of minced leaves were mixed with 1000 ml of distilled water. The mixture was boiled for 20 min, followed by incubation at room temperature for 24 h. The mixture was filtered, and the residue was subjected to a second extraction by incubating with an additional 1000 ml of distilled water for 24 h. After filtration, the combined filtrates were dried in an oven at 40°C for 72 h. The resulting dried extract was reconstituted in distilled water to a final volume of 200 ml.

1.2. Ultrasonic-assisted ethanolic extraction

The extraction process was performed according to Oroian et al. (2020) with some modifications. Approximately 1000 ml of 70% ethanol was added to 200 g of minced leaves. The mixture was sonicated in an ultrasonic water bath for 30 min. After filtration, the remaining plant material was subjected to a second 30-min sonication with an additional 1000 ml of 70% ethanol to maximize extraction yield. The combined filtrates were then dried in an oven at 40°C for 72 h. The resulting dried extract was reconstituted in distilled water to a final volume of 200 ml.

2. Bacterial Strains and Growth Conditions

The enzymatic solution was obtained from a bacterial strain previously isolated and identified as *Bosea thiooxidans* strain UAB7 (MN904936) (Yaseen, 2020). The cellulolytic bacteria were prepared in nutrient broth medium (0.5% yeast extract, 0.5% peptone, and 1% glucose) at 30°C for 24 h. The cellulolytic microorganism isolates were used to inoculate 100 ml liquid medium, 10 g CMC-Na (Mw, 1200), 10 g peptone, 0.5 g $MgSO_4$, 1 g KH_2PO_4, 1 g Na_2HPO_4 and 1000 ml water, pH 7.0 at 37°C. After 30 h of culture, the broth was separated by centrifugation (8,000 g, 10 min) at 4°C and the supernatant was collected (Yaseen, 2020).

3. Measuring Enzymatic Activities in the Cellulolytic Bacterial Supernatant

The ability of *B. thiooxidans* to secrete extracellular hydrolytic enzymes was determined. Crude suspensions extracts were used to detect

cellulase, endoglucanase, amylase and protease activities under laboratory conditions. The cellulase, endoglucanase and amylase activities were evaluated by determining the quantity of reducing sugars released from the following substrates: filter paper, amorphous cellulose (CMC), and starch respectively. The dinitrosalicylic (DNS) method was applied according to Zhang et al. (2009). One unit of enzyme activity is evaluated as the quantity of enzyme that frees 1 µmol reducing sugars (glucose), for each one ml per h.

Protease activity of the cultures supernatants of the selected isolates was measured according to the techniques described by Pokhrel et al. (2014). The crude enzyme extract (1 ml) was incubated with 2 ml of 1% casein solution and 0.1 M phosphate buffer (pH 7.0) at 50°C for 1 h. The reaction was stopped by the addition of 3 ml of 10% trichloroacetic acid. One ml of this solution was mixed with 2.5 ml of 0.5 M sodium carbonate and incubated at room temperature for 30 min. One milliliter of Folin-Ciocalteu reagent was added and incubated again for 15 min at room temperature before being measured at 660 nm. One unit of enzyme activity was defined as the amount of enzyme needed to release 1 µmol of tyrosine per min.

4. Enzyme-Assisted Extraction

Extracellular enzymes containing supernatant from *B. thiooxidans* were prepared as described above. Enzymatic solution (500 ml) was added to 200 g of minced leaves, then mixed and adjusted to pH 7.0. The solution was then shaken in a reciprocating shaker for 48 h at 37°C. Then, the solution was filtered through Whatman filter paper and dried in an oven at 40°C for 72 h. The obtained dried film was reconstituted in distilled H_2O to a final volume of 200 ml.

7. Polyphenols Determination by HPLC

Polyphenols were determined by HPLC analysis (Agilent 1260 series). The separation was carried out using Eclipse C18 column (4.6 mm x 250 mm i.d., 5 µm). The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate of 0.9 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0–5 min (80% A); 5-8 min (60% A); 8-12 min (60% A); 12-15 min (82% A); 15-16 min (82% A) and 16-20 (82%A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 5 µl for each sample solution. The column temperature was maintained at 40°C.

8. Determination of Total Phenolic

Total phenolic content was determined by the method of Mohammed and Manan (2015) with some modification, an aliquot extract of 100 µl was mixed with 300 µl of Folin-Ciocalteu reagent and 3 ml of distilled water, incubated for 10 min at room temperature then 1 ml of 20% w/v sodium carbonate (Na_2CO_3) was added to the mixture and incubated in the dark for 2 h. The absorbance was determined at 765 nm. A blank was prepared with

distilled water instead of an aliquot extract. Gallic acid standard curve was first prepared from 0-200 mg/l and total phenolic content was expressed in mg gallic acid equivalent (GE)/g fresh weight.

9. Determination of Total Flavonoid

Total flavonoid content was measured according to the method described by Kumar et al. (2015); an aliquot of 0.5 ml sample extract was mixed with 100 µl of 10% aluminum chloride, 100 µl of 1 M potassium acetate and 2.5 ml of deionized water. The mixture was kept for 30 min at room temperature and the absorbance was measured at 415 nm. Quercetin standard curve was prepared (0–50 mg/L). Total flavonoids content was expressed as mg quercetin equivalents (QE)/g fresh weight.

10. Determination of Antioxidant Activity

Preparation of plant extract, 10 ml of 50% ethanol was added to 1 g of fresh leaves and sonicated for 30 min, left in the dark at room temperature for 48 h to allow for extraction, then filtrated and the filtrate used for the DPPH scavenging assay. The assay procedure described by Baliyan (2022) with some modifications, 0.5 ml of sample extract was added to 0.5 ml of DPPH (60 µM in absolute ethanol) and left in the dark for 40 min. The absorbance of the resulting mixture was measured at 517 nm.

$$\% \text{ of antioxidant activity} = [(Ab - As) \div Ab] \times 100$$

where: Ab, absorbance of blank; As, absorbance of sample (Baliyan, 2022).

11. Determination of Plant Hormones by HPLC

The HPLC Isocratic separation was achieved using an C18 reversed-phase column (150 × 4.6 mm i.d.; 5 µm) and the mobile phase acetonitrile and acidic water (0.01% H_3PO_4) in the ratio of 60:40, Separations were carried out at room temperature with a flow rate of 1 ml/min and an injection loop of 20 µl. Quantitative analysis was performed at 206 nm.

12. Determination of Antibacterial Activity

The effect of the three different extracts on pathogenic bacteria was measured using the triphenyl-tetrazolium chloride (TTC) assay following the methodology of Eloff (1998). The gram-positive bacteria *Bacillus subtilis* and two gram-negative bacteria *Pseudomonas aeruginosa* and *Escherichia coli* were chosen for this test. These bacteria were prepared in liquid nutrient broth media with 1.6×10^8 cfu/ml bacterial density.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the three different plant extracts were determined. Original stock solutions of plant extracts were prepared at 100 mg of plant extract/ml with distilled water. Each stock solution was two-fold diluted to obtain final concentrations of 0, 1.5, 3.12, 6.25, 12.5, 25, 50 and 100 mg/ml. Fifty µl of each concentration was added to the wells of a sterile 96-well µl plate. Fifty 50 µl of bacterial suspension in nutrient broth medium was

added to serially diluted plant extract. The final volume in each well was 100 µl. The contents of each well were mixed well and incubated for 24 h at 30°C. After incubation period 10 µl/well of 2,3,5- triphenyl tetrazoliumchloride (TTC 20 mg/ml) was added and incubated under appropriate cultivation conditions for 30 min in the dark. The development of pink coloration in a well indicated bacterial growth due to TTC and the absence of the color was taken as inhibition of bacterial growth. Positive controls were wells with a bacterial suspension in nutrient broth medium. Negative controls were wells with growth medium and plant extract. All measurements of MIC values were repeated in triplicate. The MIC value was noted at the well where no color was manifested. Bacteria from each well of the microplate were sub-cultured on a nutrient agar plate; the level of dilution, where no bacterial growth on the nutrient agar plate was observed, was noted as the MBC value.

13. Nematode Inoculums

13.1. Culture preparation

Preparation of nematode inoculum to initiate and propagate pure stock culture of the root-knot nematode (*Meloidogyne incognita*) (Kofoid and White) Chit-wood, galled roots of highly infected eggplants (*Solanum melongena*) were collected. Single egg mass was used to inoculate eggplants grown under greenhouse conditions. The identification of females' perennial pattern was done according to Taylor et al. (1955). Two months later, re-inoculation on new seedling of eggplants continuously was carried out for preparing pure egg mass culture of *M. incognita.* J2s were used for all the subsequent tests (Jenkins, 1964).

13.2. Determination of nematicidal activity

Effect of *L. camara* leaf extracts on juvenile (J2) mortality of *M. incognita*. The mortality test was conducted under in vitro conditions. One ml of nematode suspension containing 50 freshly hatched juveniles of *M. incognita* was added to constant volume of *L. camara* leaf extracts (aqueous, ultrasonic assisted ethanolic and enzyme-assisted extraction) for the test, desired concentrations (25, 50 and 75 of leaf extracts into petri dishes (80 mm) and 50 freshly hatched second stage larvae of *M. incognita* in 5 ml distilled as control. All dishes were incubated in an incubator at (25±2°C). After 24, 48 and 72 h the juveniles were counted for mortality and non- mortality under stereoscope microscope. The death of nematodes was confirmed by keeping them in tap water for 24 h. The percentage mortality was worked out from an average of three replicates. The percentage of juvenile mortality was calculated using Abbott's formula (Abbott, 1925) as follows:

$$\text{Mortality}(\%) = \frac{\text{Numberofdead juveniles in thetreatment}}{\text{Total number of juveniles}} \times 100$$

Based on laboratory experiment, the data were recorded as larval mortality (dead or alive).

14. Determination of Germination Percentage and Seed Vigor Seedling Index

The seed germination experiment was conducted to study the effect of the three different extracts of *L. camara* on wheat (*Triticum aestivum* L.) seeds germination. The three extracts were diluted with distilled water to obtain concentrations of 5, 10, 15, 20, and 25%. The seeds germination test was carried out by placing 20 wheat seeds on a petri plate spread with filter paper. Subsequently, 10 ml of each concentration of the three extracts was added to the plate, which was then covered with a blotting paper to prevent external interference. The plate was incubated at 25ºC for 7 d. The number of germinated seeds and length of radicle and plumule were recorded. The results obtained were compared with the control, which consisted of seeds treated with sterile water.

The germination percentage (GP) was calculated using the following formula:

$$GP(\%) = \frac{The\ number\ of\ germinated\ seeds}{the\ number\ of\ total\ seeds} \times 100$$

Seedling vigor index (SVI) was calculated by using the formula below as suggested by Abdul-Baki and Anderson (1973).

Vigor Index (SVI) = Germination (%) × Seedling length (cm)

15. Statistical Analysis

Statistical analysis was conducted based on triplicate measurements with one-way ANOVA and subsequent Duncan's multiple range test using SPSS 17.0 program. Statistically significant differences (p<0.05) were identified between the control and experimental groups.

RESULTS

1. Characterization of Enzymatic Activities in the Bacterial Supernatant

B. thiooxidans a high-performing bacterium strain producing different types of cellulases and hemicellulases, was evaluated for production of global cellulase, endoglucanase, amylase and protease enzymes by determining their activities. The activities of cellulase, endoglucanase, and amylase were found to be 0.19, 0.45, and 0.85 mg glucose/ml/h, respectively, while the activities of protease were recorded to be 2.4 mg/ml (Fig. 2).

2. Polyphenols Profile of *L. camara* Leaf extracts

Fig. (3) summarizes polyphenols concentrations in *L. camara* leaf extracts. The results demonstrate a quantitative variability in the polyphenol profile among the three types of extracts. The aqueous extract exhibited the highest levels of these compounds, including kaempferol (508.74 µg/ml), apigenin (58.44 µg/ml), daidzein (55.91 µg/ml), syringic acid (40.20 µg/ml),

catechin (22.89 µg/ml), chlorogenic acid (21.71 µg/ml), ellagic acid (9.48 µg/ml), hesperetin (7.29 µg/ml), and methyl gallate (2.08 µg/ml), respectively.

The ultrasonic-assisted ethanolic extract followed the aqueous extract, with the most abundant polyphenols being quercetin (1636.08 µg/ml), naringenin (13.52 µg/ml), pyrocatechol (8.35 µg/ml), cinnamic acid (6.22 µg/ml) and coumaric acid (1.90 µg/ml). Finally, the enzyme-assisted extract had the highest levels of caffeic acid (18.69 µg/ml) and rutin (3.91 µg/ml), respectively.

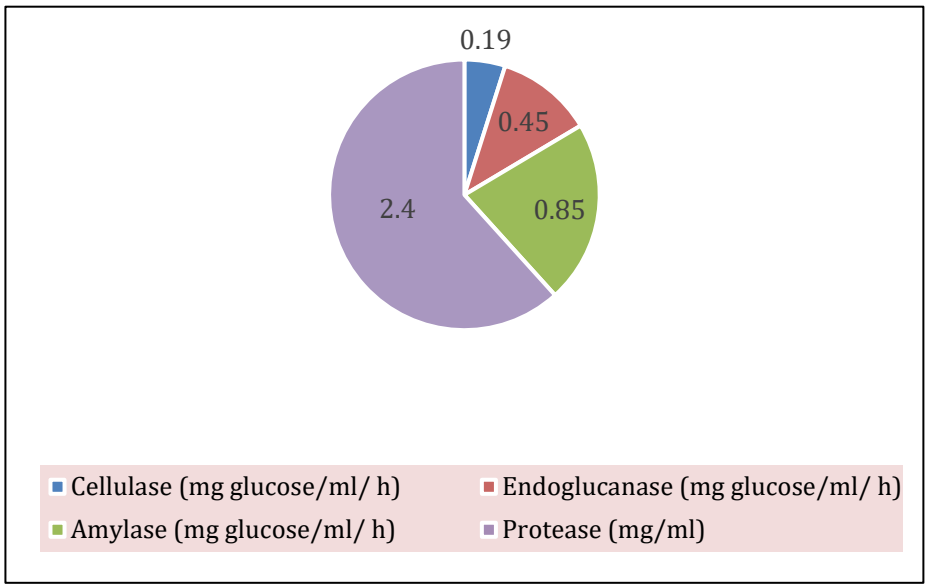

Fig. (2). Enzymatic activities in bacterial supernatants.

3. Total Phenolics, Flavonoids, and Antioxidant Activity of *L. camara* Leaf Extracts

Table (1) provides that the aqueous extract of *L. camara* leaves had the highest levels of total phenolics (17.42 mg QE/g), and flavonoids (7.61 mg GE/g), and exhibited the highest antioxidant activity (91.19%). Both ethanolic and enzyme-assisted extracts showed significantly lower levels of these compounds and antioxidant activity compared to the aqueous extract.

4. Phytohormones Profiles in *L. camara* Extracts

Fig. (3) illustrates that the extraction methods affected the phytohormone profiles of *L. camara* leaves. The enzyme-assisted extract contained the highest concentration of GA_3 (10.55 µg/ml) and a considerable amount of 6-benzyle adenine (9.87 µg/ml), suggesting potential for growth-promoting applications. Surprisingly, the aqueous extract had the highest content of 6-benzyle adenine. Interestingly, the ultrasonic-assisted ethanolic extraction appears most efficient for ABA (10.41 µg/ml).

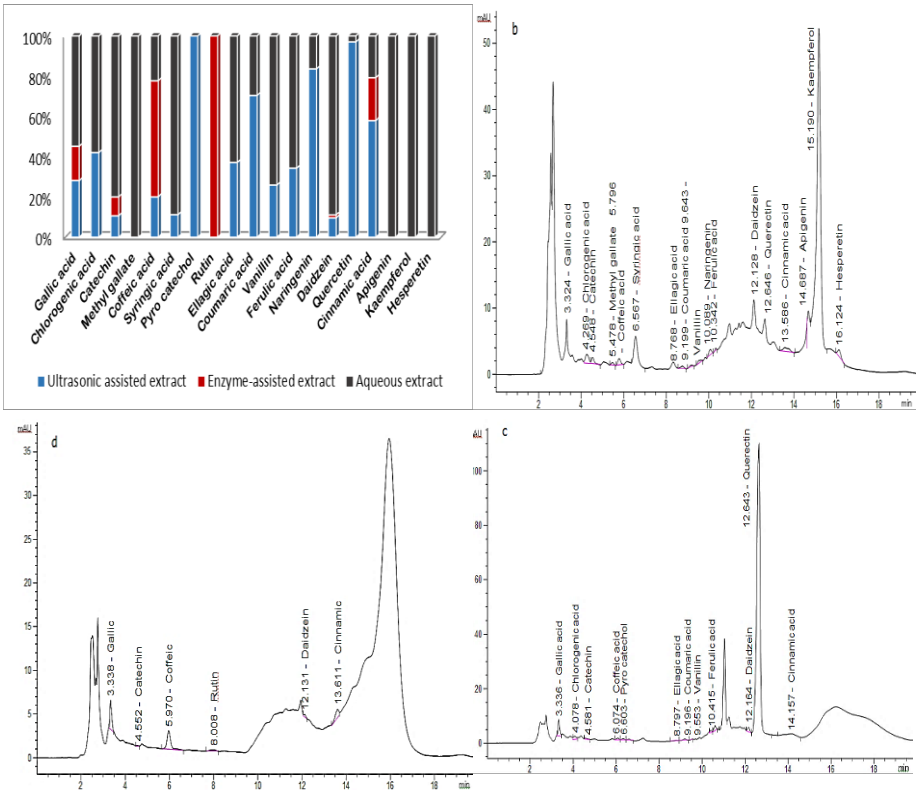

Fig. (2). Polyphenols of *Lantana camara* leaves **a.** concentration of polyphenols, **b.** HPLC chromatogram of aqueous extract, **c.** HPLC chromatogram of ultrasonic-assisted ethanolic extract and **d.** HPLC chromatogram of enzyme-assisted extract.

Table (1). Total phenolics, flavonoids and antioxidant activity of *Lantana camara* leaf extracts

Extract	Total phenolics (mg QE)/g)¶	Total flavonoids (mg GE)/g)¶	Antioxidant activity (%)
Aqueous	17.43[a]	7.61[a]	91.19[a]
Enzyme-assisted	8.38[c]	3.45[c]	29.74[b]
Ultrasonic-assisted	10.23[b]	4.10[b]	16.21[b]
F-value	112.75	182.47	81.87
P-value	<0.001	<0.001	<0.001

QE and GE refer to quercetin equivalent and gallic acid equivalent, respectively. The values represent the means of three replicates. Different letters within the same column indicate significant differences according to Duncan's multiple range tests.

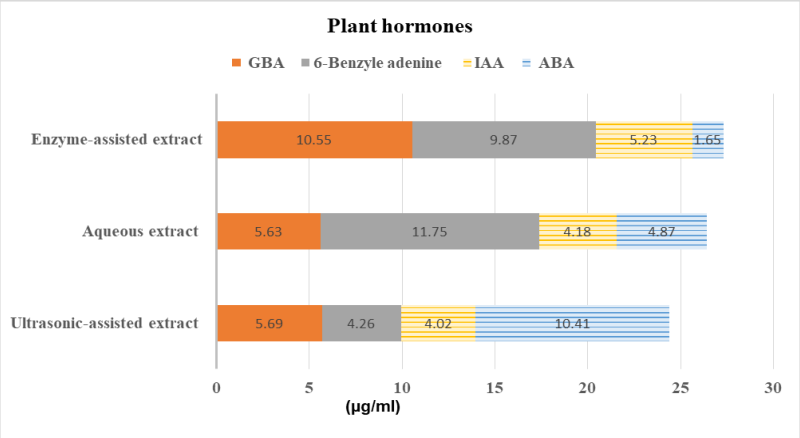

Fig. (3). Phytohormones profiles in *Lantana camara* extracts.

5. Determination of MIC and MBC of the Three Bioactive Leaf Extract of *L. camara*

Fig. (4) shows MIC and MBC of three bioactive leaf extracts from *L. camara* against the test organisms. MIC refers to the lowest concentration of an extract that inhibits the visible growth of a microorganism based on metabolic activity, while MBC refers to the lowest concentration at which the extract kills the microorganism. The MIC and MBC values were in the range of 3.12-50%, with the lowest MIC and MBC values of 3.12 and 25%, respectively in each case recorded against *B. subtilis*, *P. aeruginosa* and *E. coli*. The enzyme assisted extracts of *L. camara* particularly had better antibacterial activities in comparison to other extracts. The high MIC and MBC values are an indication of the lack of efficacy of the plant extracts against the test bacteria.

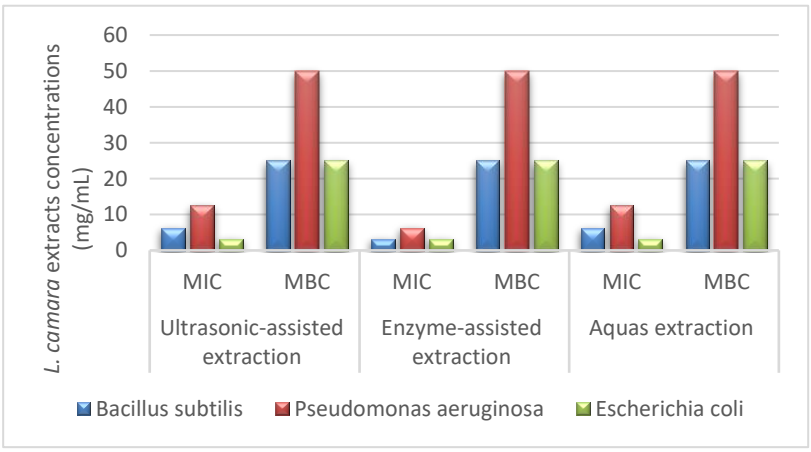

Fig. (4). Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the three bioactive leaf extracts of *Lantana camara*.

6. Bio Efficacy of *L. camara* Leaf Extracts against *M. incognita*

Data presented in Table (2) show that *L. camara* leaf extracts exhibited varying degrees of nematicidal activity against *M. incognita* (root-knot nematode). All extracts concentrations caused nematode mortality compared to the control. Furthermore, using higher concentrations and longer times of exposure to extract led to increased mortality in *M. incognita* larvae. The maximum mortality of *M. incognita* J2 (100%) was recorded in 75 the ultrasonic-assisted concentration of *L. camara* leaf extracts at 48 and 72 h of exposure time followed by 50 ultrasonic-assisted concentration at 72 h recorded (94%), while the enzyme-assisted extract at 75 concentration recorded 84% whereas minimum (22 and 30%) was recorded at 25 concentration in aqueous and enzyme-assisted extract after 24 h of exposure time followed by aqueous extract was recorded (40%) in concentration 50 after 48 h treatments exhibited natural nematicidal potential to varying degree. The result showed significant juvenile mortality potential of plant extract against *Meloidogyne* juveniles.

Table (2). Effect of *Lantana camara* leaf extracts on juvenile (J2) mortality of *Meliodogyne incognita.*

Extract	Conc.	Time					
		24 h		48 h		72 h	
		No. of immobile juvenile	Mortality (%)	No. of immobile juvenile	Mortality (%)	No. of immobile juvenile	Mortality (%)
Aqueous	**25**	11.00± 1.45^{a}	22	20.00± 1.73^{a}	40	28.00± 1.15^{a}	56
	50	21.00± 1.20^{c}	42	29.00± 0.67^{b}	58	33.00± 0.88^{b}	64
	75	24.00± 1.45^{c}	48	30.67± 1.45^{b}	62	39.00± 0.58^{c}	78
Enzyme-assisted	**25**	15.00± 0.88^{b}	30	22.00± 1.45^{a}	44	31.00± 1.15^{ab}	62
	50	21.00± 1.76^{c}	42	31.00± 1.00^{b}	62	34.00± 1.45^{b}	68
	75	28.00± 1.15^{d}	56	36.00± 1.45^{c}	72	41.67± 2.03^{cd}	84
Ultrasonic-assisted	**25**	29.00± 1.73^{d}	58	35.00± 1.15^{c}	70	45.00± 1.15^{de}	90
	50	37.00± 0.58^{e}	76	45.00± 1.15^{d}	90	47.00± 1.76^{ef}	94
	75	46.00± 0.33^{f}	92	50.00± 0.00^{e}	100	50.00± 0.00^{f}	100
Control		0	0	000	0	00	0
F-value		72.566		63.950		38.198	
P-value		<0.001		<0.001		<0.001	

The values represent mean ± SE. Different letters within the same column indicate significant differences ($p < 0.05$) according to Duncan's multiple range test.

7. Effect of *L. camara* Leaf Extracts on Seedling Vigor Index and Germination Percent of Wheat

The effect of different concentrations of *L. camara* leaf extracts on SVI and germination percent (G %) of wheat was evaluated on the seventh day from germination. Data in Table (3) reveal that SVI of wheat was significantly decreased at all concentrations of aqueous extract and the reduction was more prominent at the highest concentration. This result suggested the presence of the allele-chemicals in aqueous plant extract. On the other hand, Enzyme-assisted extract significantly promoted SVI by 10.77, 27.04 and 32.7% at 5, 10 and 15% concentrations, respectively, compared to the control, highlighting potential growth-enhancing compounds. Ultrasonic-assisted extract had a lower vigor index compared to the control. The plant extracts at concentrations greater than 15% reduced significantly the total seedling vigor index. A similar effect was observed on germination percentage for all three extracts.

Table (3). Wheat seedling vigor index (SVI) and germination percent (G%) as affected by *Lantana camara* leaf extracts.

Extract	Conc.	SVI	G %
Aqueous	0	506.170^{cd}	84.375
	5	592.170^{ab}	95.000
	10	258.670^{f}	80.000
	15	130.670^{gh}	70.000
	20	25.667^{ij}	70.000
	25	0.000^{j}	0.000
Enzyme-assisted	0	506.170^{cd}	84.375
	5	566.670^{abc}	85.000
	10	546.000^{bc}	70.000
	15	618.000^{a}	90.000
	20	181.330^{g}	80.000
	25	74.333^{hi}	70.000
Ultrasonic-assisted	0	506.170^{cd}	84.375
	5	477.330^{d}	80.000
	10	397.330^{e}	80.000
	15	376.830^{e}	85.000
	20	245.330^{f}	80.000
	25	89.167^{hi}	75.000
F-value		24.07	
P-value		<0.001	

The values represent the means of three replicates. Different letters within the same column indicate significant differences according to Duncan's multiple range test.

DISCUSSION

Extracting bioactive compounds from plants like *L. camara* requires careful consideration, due to the impacts of extraction method both the

quantity and properties of the extracted molecules. This study compared three techniques: the classical aqueous extraction, and the innovative ultrasonic and enzyme-assisted approaches. For enzyme assisted approach, *B. thiooxidans* was used as a natural source for hydrolysis enzymes. Microbes like *B. thiooxidans*, known for their enzyme diversity and thermo-stability, hold vast potential for industrial applications (Nigam, 2013; Parte et al., 2017 and Liu and Kokare, 2023). In fact, Houfani et al. (2021) found that *Bosea* sp. FBZP-16 efficiently produced a variety of cellulases and hemicellulases, with their activity peaking at specific temperatures, shaking speeds, and nutrient concentrations. They added that *Bosea* sp. FBZP-16 shows significant promise as a natural enzyme factory for utilizing lignocellulosic biomass in biofuel production.

Polyphenols are attracting attention for their potential health benefits and antioxidant properties. This has led to the development of "functional foods" enriched with these compounds. Various extraction techniques are being explored to recover polyphenols from diverse plant sources. Hot water maceration yielded the highest amounts of polyphenols, total phenolics, and flavonoids compared to ultrasonic and enzyme-assisted methods, exhibiting the strongest antioxidant activity. Analysis of the extracts revealed that the aqueous extract is particularly rich in kaempferol. This finding suggests that this extraction method could be a powerful and practical way to obtain valuable flavonoid. Kaempferol (3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one) belongs to the subclass of flavones within the flavonoid family (Chen et al., 2023). Notably, the presence of three hydroxyl groups in kaempferol likely contributes to its abundance in the aqueous extract. These hydroxyl groups can readily form hydrogen bonds with water molecules, facilitating its extraction using this aqueous method. Similarly, the ultrasonic-assisted ethanolic extract emerged as a potent source for obtaining quercetin (another member of the flavonoid family). Quercetin, a potent polyphenolic flavonoid, exhibits strong antioxidant activity by maintaining oxidative balance through its effects on glutathione, signal transduction pathways, enzymatic activity, and scavenging reactive oxygen species (Xu et al., 2019). Also, quercetin contributes to intramolecular hydrogen bonds within the quercetin molecule itself (Protsenko, 2014), reducing the availability in aqueous extract and facilitating ethanolic extraction. Biological extraction using enzymes might lead to altered polyphenol profiles compared to other methods. This could involve a decrease in certain polyphenols, potentially due to enzyme activity converting them into new compounds with potential biological activity (Vilas-Franquesa et al., 2024).

The aqueous extraction method effectively preserves the phytochemical and antioxidant potential of *L. camara* leaves. However, ethanolic extract possesses considerable antioxidant activity, warranting further investigation. Phenolic and flavonoid content analysis plays a crucial role in understanding the antioxidant potential of plant extracts. Flavonoids

are recognized for their remarkable ability to combat oxidative stress. These bioactive compounds function as versatile warriors against free radicals, acting as reducing agents, scavengers, and metal chelators (Sousa, et al., 2015).

Different extraction methods of *L. camara* leaves also affected their phytohormone profiles, dictating potential applications. Enzyme-assisted extracts boast high growth-promoting gibberellic acid, while aqueous extracts reign in cytokinin-rich 6-benzyle adenine useful for stimulating fruit richness by stimulating cell division and postharvest storage. Ultrasonic extracts hold both auxin for root development and stress-regulating abscisic acid. Understanding this complex interplay paves the way for targeted applications in growth promotion, stress management, and sustainable agriculture.

Antibacterial activity of extracts against *B. subtilis, P. aeruginosa* and *E. coli* was evaluated by determination their MIC and MBC. The high MIC and MBC values is an indication of lack of efficacy of the plant extracts against the test bacteria. MIC refers to the lowest concentration of an extract that inhibits the visible growth of a microorganism based on metabolic activity, while MBC refers to the lowest concentration at which the extract kills the microorganism. In our study the enzyme-assisted extracts consistently displayed superior antibacterial activity compared to the other two methods. This aligns with Millet et al. (2012), where the fermented extract of onion, lacking the standard flavonoid composition, exhibited the highest activity. They suggested that metabolites of onion compounds generated through enzymatic processes may be more potent than their precursors. The study of Thorat et al. (2021) suggested a link between the presence of secondary metabolite "triterpene" in *L. camara* extracts and the observed antimicrobial activity. Additionally, Dubey and Padhy (2013) as well as Saraf et al. (2011) highlighted the broad antimicrobial spectrum of *L. camara* phytochemicals, suggesting potential for novel drug development.

The nematocidal potential of the three extracts was also determined. Results revealed that higher concentrations and longer durations significantly increased mortality across all extracts. These results agree with the findings of Akhtar and Mahmood (1994), Ali et al. (2001), Shaukat and Siddiqui (2001) and Bhuyan (2017), who reported that the highest concentrations of leaf extracts of *L. camara* caused maximum mortality of J2 of *M. incognita* under in vitro conditions. Among all the concentrations of leaf extract of *L. camara* tested, 75% concentration at 72 h was found effective in controlling *Meloidogyne* juveniles. This result agrees with the result obtained by Akhtar and Mahmood (1994), who reported that extracts from leaves and root of Mexican marigold and leaves of *Lantana* reduced the hatching of *M. incognita* eggs significantly. The nematicidal activity of *L. camara* against juveniles of *Meloidogyne* spp. has also been reported by many authors (Shaukat and Siddiqui, 2001; Qamar et al., 2005 and Begum et al., 2008). The mortality of

juveniles might be due to nematicidal chemicals present in the leaf extract as *L. camara* contains camaric acid and olenolic acids which may have larvicidal properties. Notably, ultrasonic-assisted extracts showed the best nematocidal activity followed by enzyme-assisted then aqueous extraction. This method of extract preparation probably enabled an increase both in the availability and activity of allelochemical compounds that antagonize nematode growth. According to findings of Bordoloi et al. (2021) and Malahlela et al. (2021) the nematocidal allelochemicals from *L. camara* were present in all the plant tissue. Therefore, *L. camara* extracts can be an important ingredient in the suppression of plant diseases.

Allelopathic plants can be widely used in bio-farming considering their potential role in the improvement of seed germination. The term allelopathy refers to the adverse or beneficial effect of one plant on the other because of producing and releasing metabolites to the environment (Findura et al., 2020). In this study *L. camara* leaf extracts were used for wheat seed dressing to determine their effects on seedling vigor index. Across all extraction methods, aqueous extracts suppressed wheat SVI, suggesting allelopathic compounds. Conversely, enzyme-assisted extracts significantly boosted seedling growth, potentially due to the presence of gibberellic acid growth-promoting compounds. Potentially due to using ultrasonic-assisted extracts had a neutral to slightly negative effect, both indole-3-acetic acid and abscisic acid. Similarly, Radwan et al. (2019) showed that higher concentrations of aqueous extract from *Calotropis procera* L. (7 and 10%) significantly reduced germination percentage, radicle length and plumule length of wheat as compared to control. Mistica et al. (2023) found that the concentration of the aqueous leaf extract of *L. camara* is directly proportional to the growth inhibitory effects on seed germination and root and shoot elongation. Gindri et al. (2020) highlighted that lantadene A and B are the possible compounds conferring the herbicidal properties in aqueous extract, and the *L. camara* extract has a potential to be used in the development of a new environmentally friendly herbicide.

CONCLUSION

This study revealed that *L. camara* leaf extracts, particularly those obtained through ultrasonic and enzyme-assisted methods, demonstrate stronger antibacterial activity against harmful pathogens. Furthermore, these extracts exhibit promising nematicidal properties, suggesting their potential to become revolutionary alternatives to synthetic pesticides in agriculture. Notably, ultrasonic and enzyme-assisted extracts also act as growth promoters for wheat seedlings, showcasing their potential to enhance agricultural productivity sustainably. While the aqueous extract holds higher levels of phytochemical and antioxidant activity, it highlights the crucial role of extraction methods in targeting specific bioactive compounds. This opens

avenues for further exploration, allowing us to tailor extraction processes to specific needs in pest management, agricultural practices, and potentially even human health applications like wound healing or antimicrobial treatments. Further research into these extracts and their constituent compounds could pave the way for the development of novel, sustainable, and eco-friendly solutions that address various challenges while safeguarding the environment and human health.

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التقييم المقارن للمواد الفيتوكيميائية ونشاط التضاد الميكروبي والنشاط المضاد للأكسدة لمستخلصات أوراق نبات اللانتانا كمارا

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نبات اللانتانا كمارا هو عبارة عن شجيرة سريعة الانتشار وهو مستوطن في المناطق الاستوائية في أفريقيا وآسيا وأمريكا. هذه الدراسة بحثت في فوائدها المحتملة من خلال استخلاص المكونات النشطة من أوراقها باستخدام طرق مختلفة بما في ذلك الاستخلاص التقليدي (الاستخلاص المائي) وغير التقليدي (الاستخلاص الإيثانولى بالموجات فوق الصوتية والاستخلاص القائم على استخدام الإنزيمات). تم تحديد خصائص البوليفينول والهرمونات النباتية للمستخلصات بواسطة التحليل الكروماتوجرافي HPLC. تم تقييم النشاط المضاد للنمو للمستخلصات من خلال دراسة تأثيرها ضد البكتيريا والنيماتودا. والجدير بالذكر أن المستخلص القائم على استخدام الإنزيمات كان له نشاط مضاد للجراثيم أفضل مقارنة بالطرق الأخرى. بالإضافة إلى ذلك، أظهرت المستخلصات درجات متفاوتة من النشاط المضاد للنيماتودا، مما يدل على فعالية كبيرة ضد *Meloidogyne*. علاوة على ذلك، تم تقييم النشاط البيولوجي العام للمستخلصات باستخدام الاختبار الحيوي لإنبات البذور. ومن المثير للاهتمام أن المستخلص الإيثانولى بالموجات فوق الصوتية وبمساعدة الإنزيم عزز بشكل كبير نمو شتلات القمح. في المقابل، أظهر المستخلص المائي أعلى نشاط للمواد الكيميائية النباتية ومضادات الأكسدة مقارنة بالمستخلصات بالموجات فوق الصوتية والمستخلصات الإنزيمية. يشير هذا إلى أن طرق الاستخلاص المختلفة قد تستهدف مركبات نشطة بيولوجيًا مختلفة ذات تأثيرات بيولوجية متنوعة. في الختام، تشير هذه الدراسة إلى أن مستخلصات نبات اللانتانا كمارا تمتلك خصائص واعدة مضادة للبكتيريا ومبيد للديدان ومضاد للأكسدة. وهذا يفتح سبلاً لمزيد من البحث والتطوير المحتمل للبدائل الطبيعية لمختلف التطبيقات.