



# Comparative Phytochemical Profiling of *Psophocarpus tetragonolobus* (L.) D.C. Seed Extracts for Effective Storage of Cowpea Seeds

S. Kavitha<sup>1</sup>, J. Renugadevi<sup>1</sup>, P.R. Renganayaki<sup>1</sup>, M. Suganthy<sup>1</sup>,  
P. Meenakshi<sup>1</sup>, K. Raja<sup>1</sup>, K. Madhan<sup>1</sup>

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## ABSTRACT

**Background:** Winged bean (*Psophocarpus tetragonolobus* (L.) D.C.), is unique legume which has numerous phytochemical with distinct properties. So, this research work was performed to explore and quantify the bioactive compound from different extracts of winged bean seeds through GC-MS and to assess the bio-efficacy on cowpea seed to protect them against deterioration and bruchid.

**Methods:** The bioactive compounds of winged bean seed extracts (from different solvents, viz., water, ethanol and hexane) were analysed by GC-MS and the bio-efficacy of identified superlative extract on maintaining the quality of cowpea seed var. VBN 3 was done using accelerated ageing method.

**Result:** The GC-MS results showed the attendance of 30 different compounds in different extracts of the winged bean seeds. Of the different compounds eluted from extract, the ethanolic seed extract revealed the existence of distinct phytochemicals such as d-mannitol, 1,4-anhydro-(1.59%), diethyl phthalate (27.21%), n-hexadecanoic acid (4.54%), 9,12-octadecadienoic acid (Z,Z)- (9.71%), oleic acid (14.16%), octadecanoic acid (4.89%), 9,12,15-octadecatrienoic acid and 2-phenyl-1,3-dioxan-5-yl ester (1.05%) which possess significant antioxidant and insecticidal properties. Among the different concentration of ethanolic extract, cowpea seeds treated with 2.5% of ethanolic seed extract of winged bean showed the highest germination (94%) and vigour index I (4436) compared to other treatments while the control recorded lowest germination (88%) and vigour index (3572) at initial day. As ageing progresses, seed germination percent and vigour index of 2.5% ethanolic extract treated seeds dropped to 63% and 1845 while, it is 42% and 953 in case of untreated seeds. The bio-efficacy study of ethanolic extract against bruchid revealed the LD<sub>90</sub> value (2.5%) of winged bean seed extract. This study clearly shows that ethanolic extract of winged bean seeds have many bioactive compounds with distinct properties that maintain the seed quality under storage.

**Key words:** Bruchid, GC-MS, Phytochemical, *Psophocarpus tetragonolobus*, Winged bean.

## INTRODUCTION

Winged bean [*Psophocarpus tetragonolobus* (L.) D.C.] also known as tropical soybean and poor man's food, belongs to family Fabaceae. It is an underutilised diploid legume which is widely cultivated in Asia and Africa (Mohanty *et al.*, 2020). Winged bean seeds contain polyphenols, tannins, phytic acid, trypsin inhibitor, chymotrypsin inhibitor, protease inhibitor, lectins, amylase inhibitors, phytates and other phenolic compounds (Janson *et al.*, 2020). It also contains erucic acid and polyunsaturated fatty acids.

Despite all these, we lack the knowledge on their bioactive compounds and its potential. Thus, exploration of winged bean seed phytochemicals and its potential are of utmost important for its utilization in seed quality maintenance as quality seeds are vital input for the establishment of healthy crop. The loss of pulse seeds during storage is a major issue. In India, a projected annual loss owing to seed deterioration during storage amounts to 25% of the produced crop (Mahjabin, 2015).

Apart from rapid deterioration of seed vigour caused by ageing, quality is also impacted by insect during storage. The primary pest of cowpea storage is the pulse beetle.

<sup>1</sup>Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.

**Corresponding Author:** S. Kavitha, Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India. Email: kavithasoumi@gmail.com

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Within a six-month period, it may damage 100% of the stored seeds and result in a 60% loss of grain (Kang *et al.*, 2013). To prevent seeds from deterioration, seed treatment is one of the measures. One such safe and effective seed treatment involves botanicals to maintain vigour and viability during storage. So, the present work aimed at profiling the bioactive constituents of winged bean seed extract using different solvents through GC-MS and to evaluate the efficacy of

identified preeminent extract on seed quality maintenance of cowpea under storage.

## MATERIALS AND METHODS

The experiment was done at Department of Seed Science and Technology, Tamil Nadu Agricultural University (TNAU), Coimbatore during 2019-20. Freshly harvested seeds of winged bean cv. Revathy obtained from Kerala Agricultural University (KAU), Vellanikkara, Kerala and genetically pure seed lots cowpea var. VBN 3 obtained from the National Pulses Research Centre (NPRC), Vamban were used as the base seed material for this study.

### Preparation of extract

For preparing extract, each solvent *viz.*, water, ethanol, hexane and seed powder were taken separately in stopped flask in 1:10 w/v ratio. The flasks were shaken every hour for the first 6 h and then kept aside and again shaken after 24 h for every three days and then solvents were filtered with Whatman No.41 filter paper. The filtered solvents were collected and dried using a rotary vacuum evaporator and then lyophilized to get solid extracts. The solid masses of extracts were stored at -20°C until it was used for GC-MS analysis by the following method described by Alagammal *et al.* (2012).

### Gas Chromatography- Mass Spectrum analysis

The phytochemical constituent's analysis was carried out by following the method of Hema *et al.* (2010) using the Perkin-Elmer GC Clarus SQ 8C system. It has an Elite-I, fused silica capillary column (30 mm × 0.25 mm 1D × 1 μMdf, formed of 100% Dimethyl polysiloxane) and an electron ionization device with an ionising energy of 70 eV. The carrier gas, helium (99.999%) was utilized with an injection volume of 2 μl and a constant flow rate of 1 ml/min (split ratio: 10:1); 250°C for the injector and 280°C for the ion source. The oven temperature was configured to begin at 110°C (isothermal for 2 min), rise at a rate of 10°C/min to 200°C, then 5°C/min to 280°C and ending with a 9 min isothermal at 280°C. At 70eV, 0.5s of scanning time and fragments ranging in size from 45 to 450 Da, mass spectra were recorded. The GC ran for 30 min in total. The turbomass software was used to handle mass spectra and chromatograms and it was utilized to compute the relative percentage amount of each component by comparing its average peak area to the total areas.

### Identification of components

Interpretation of the GC-MS mass spectrum was done using National Institute of Standard and Technology (NIST) database. A comparison was made between the spectra of the unknown component and the spectra of the known components in the NIST database.

### Physiological seed quality parameters

Cowpea seeds with initial viability of 88 percent were coated with ethanolic extract of winged bean seeds at various

concentrations *viz.*, 0.5, 1.5, 2.5, 3.5, 4.5 and 5.5% and then subjected to accelerated ageing. The treated and untreated seeds were kept in perforated butter paper covers and kept in accelerated ageing chamber as per the protocol described by Delouche and Baskin (1973). Seed sample from each treatment were drawn on the 2<sup>nd</sup>, 4<sup>th</sup>, 6<sup>th</sup>, 8<sup>th</sup> and 10<sup>th</sup> days and assessed for physiological seed quality parameters such as germination (%) and vigour index.

$I (VI) = \text{Germination (\%)} \times \text{Total seedling length (cm)}$

as per ISTA (2019) seed testing protocols. Winged bean seeds extract were dissolved in 100 percent ethanol to prepare solutions at different concentrations.

### Effects of the ethanolic seed extract on adult mortality of bruchid, *Callosobruchus maculatus*

Contact toxicity assay was performed with ethanolic seed extract. For each concentration, 50 g of cowpea seeds were treated with extract and air dried and taken in a plastic container and 20 pairs of newly emerged beetles at the ratio of 1:1 were released. The treatment and control was maintained separately. The readings were taken at 24 h interval for 3 days and number of insects dead was recorded. The mortality percentage was calculated by the formula given by Abott (1925).

$$\text{Corrected (\%)} \text{ mortality} = \frac{X-Y}{100-Y} \times 100$$

X= Percentage mortality in the ethanolic seed extract treated samples.

Y= Percentage mortality in the untreated check.

### Statistical analysis

An analysis of variance (ANOVA) with a factorial combination of treatments was used to analyse the experiment-derived data. The critical difference was calculated at the 0.05 probability level. Values in the percent data were converted using an arcsine before analysis (Panse and Sukhatme, 1995).

## RESULTS AND DISCUSSION

### GC-MS analysis

The active principles present in the winged bean seed extracts was identified by GC-MS (Fig 1, 2 and 3), their retention time, probability and peak area percentage are presented in Table 1, 2 and 3. Thirty distinct compounds were identified in each extract and only 20 were tabulated.

The aqueous seed extract has hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (2.93%), n-hexadecanoic acid (2.00%), oleic acid (14.86%), E,E,Z-1,3,12-nonadecatriene-5,14-diol (1.92%), 9,12-octadecadienoic acid (Z,Z)-2-hydroxy-1-(hydroxymethyl) ethyl ester (4.98%), 9-octadecenoic acid (Z)-2,3-

dihydroxypropyl ester (17.13%) at various time intervals comprising major peak area percentage.

The GC-MS analysis of the ethanolic extract of winged bean seeds showed major compounds viz., d-mannitol, 1,4-anhydro- (1.59%), diethyl phthalate (27.21%), n-hexadecanoic acid (4.54%), 9,12-octadecadienoic acid (Z,Z)- (9.71%), oleic acid (14.16%), octadecanoic acid (4.89%), 9,12,15-octadecatrienoic acid, 2-phenyl-1,3-dioxan-5-yl ester

(1.05%). From the MS chromatogram, a total of 30 compounds were identified.

While, the GC-MS analysis of the hexane seed extract indicated the presence of 2-decenal, (Z)- (2.65%), 2,4-decadienal, (E, E)- (1.53%), n-hexadecanoic acid (11.26%), 9-octadecenoic acid, (E)- (16.12%), octadecanoic acid (5.16%) and cis-13-eicosenoic acid (1.68%) were the most abundant compounds in the extract.

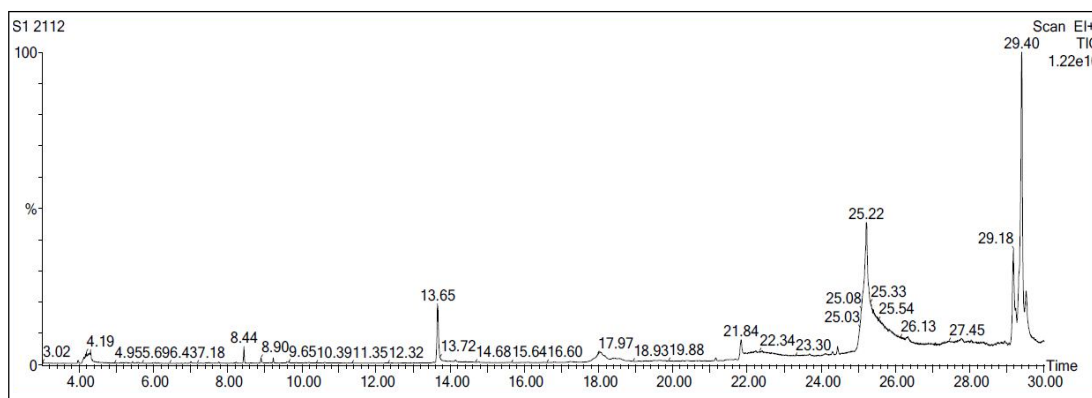


Fig 1: GC-MS chromatogram of aqueous seed extract of winged bean.

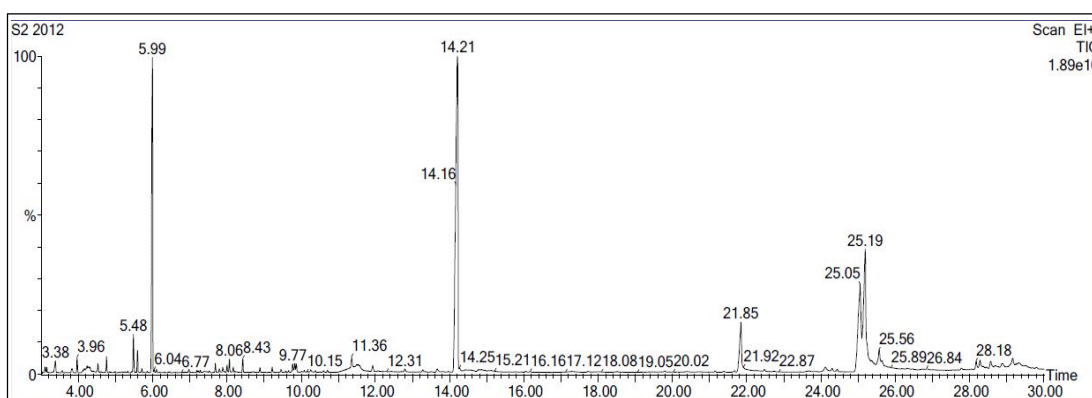


Fig 2: GC-MS chromatogram of ethanol seed extract of winged bean.

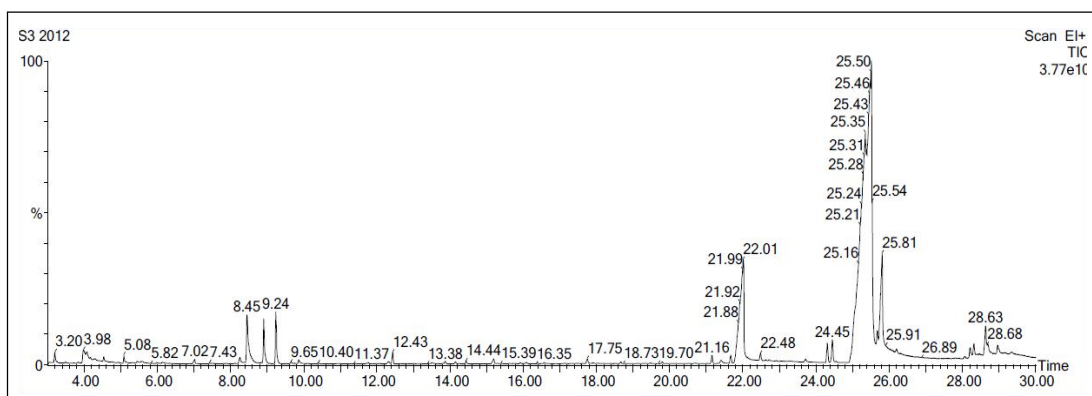


Fig 3: GC-MS chromatogram of hexane seed extract of winged bean.

The effectiveness of medicinal plants is assessed by correlating the bioactive compounds to the biological activities of the plants (Belkacem *et al.*, 2013). The phytochemicals present in the winged bean seed extract might be responsible for wide range of properties *viz.*,

antioxidant, insecticidal, larvicidal, antifungal, antibacterial, hemolytic, hypocholesterolemia, anti-androgenic and 5-Alpha reductase inhibitor (Table 4). The phytochemicals of ethanol seed extract showed the presence of distinct compounds, with noticeable

**Table 1:** The bioactive compounds from the aqueous seed extract of winged bean by GC-MS analysis.

Retention time	Compounds	Probability	Area (%)
3.1	2H-Benzo[f]oxireno[2,3-E]benzofuran-8(9H)-one,9-[[[2(dimethylamino)ethyl] amino]methyl]octahydro-2,5a-dimethyl-	9.4	0.30
4.1	Glycerin	28.6	0.69
8.4	2-Decenal,(Z)-	45.4	0.42
18.0	Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl)ethyl ester	69.6	2.93
21.8	n-Hexadecanoic acid	67.4	2.00
22.1	Eicosanoic acid	17.0	0.61
22.2	l-(+)-Ascorbic acid 2,6-dihexadecanoate	14.3	1.09
22.3	Dasycarpidan-1-methanol, acetate (ester)	20.2	0.43
22.4	Octadecanoic acid,4-hydroxy-,methylester	27.7	0.66
22.4	3-Octadecenoic acid,methyl ester	10.2	0.84
22.7	Hexadecanoic acid,1-(hydroxymethyl)-1,2-ethanediyl ester	17.0	0.41
24.4	10-Octadecenoic acid, methyl ester	6.8	0.35
25.2	Oleic Acid	24.9	14.86
26.3	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	5.1	1.92
27.4	Ethyl iso-allocholate	25.1	0.52
27.7	Ethanol,2-(9-octadecenyl)-,(Z)-	8.6	0.93
27.9	Octadecanal,2-bromo-	9.5	0.53
28.9	Pentadecanoic acid,2-hydroxy-1-(hydroxymethyl)ethyl ester	41.6	0.32
29.1	9,12-Octadecadienoic acid (Z,Z)-,2-hydroxy-1-(hydroxymethyl)ethyl ester	34.4	4.98
29.3	9-Octadecenoic acid (Z)-,2,3-dihydroxypropyl ester	18.1	17.13

**Table 2:** The bioactive compounds from the ethanol seed extract of winged bean by GC-MS analysis.

Retention time	Compounds	Probability	Area (%)
3.3	Maleic anhydride	33.1	0.51
3.9	2-Heptenal,(Z)-	41.3	0.38
4.2	1-Propanol	22.7	0.55
8.0	Acetic acid, diethoxy-,ethyl ester	26.9	0.34
8.4	2-Decenal,(Z)-	42.6	0.38
11.3	3,3-Diethoxy-1-propanol, butyl ether	12.2	0.79
11.4	d-Mannitol,1,4-anhydro-	11.6	1.59
14.2	Diethyl Phthalate	86.5	27.21
14.5	Melezitose	8.2	0.44
15.1	3-Deoxy-d-mannonic acid	29.8	0.44
21.8	n-Hexadecanoic acid	84.3	4.54
24.1	E-10,13,13-Trimethyl-11-tetradecen-1-ol acetate	5.1	0.59
25.0	9,12-Octadecadienoic acid (Z,Z)-	51.5	9.71
25.1	Oleic Acid	20.7	14.16
25.5	Octadecanoic acid	67.9	4.89
26.1	Hexadecanoic acid, ethyl ester	14.2	0.36
28.2	2-Pyrrolidinone, 1-(9-octadecenyl)-	13.9	0.47
28.5	cis-11-Eicosenoic acid	11.9	0.41
29.1	9,12-Octadecadienoic acid (Z,Z)-,2-hydroxy-1-(hydroxymethyl)ethyl ester	26.0	0.87
29.3	9,12,15-Octadecatrienoic acid,2-phenyl-1,3-dioxan-5-yl ester	6.5	1.05

properties which are required for seed, than the aqueous and hexane seed extract.

Shivasharanappa *et al.* (2018) reported that seed deterioration causes delayed germination, slower seedling growth rates, abnormal growth, reduced vigour index, diminished tolerances to adverse conditions and ultimately loss of germination ability. It is mainly due to the lipid peroxidation of seed membrane. Phytochemicals play vital roles in the free radical scavenging activities of plants. The antioxidant properties of plants are attributed to their rich phytochemical composition (Charles *et al.*, 2018).

The findings in Table 5 and 6, shows the effect of different concentrations of winged bean ethanolic seed extract on germination of cowpea seeds under accelerated ageing. Regardless of the duration of the accelerated ageing process, seeds treated with 2.5% of the winged bean seed extract recorded the highest germination (80%) and vigour index (3174) among all other concentrations. The lowest germination and vigour index (68% and 2313) was recorded in untreated seeds. It was found that the germination and vigour index germination % had considerably decreased from 91 per cent and 3994 on the initial day to 53 per cent and 1374 on the 10<sup>th</sup> day of accelerated ageing. At the 10<sup>th</sup> day of accelerated ageing, the seeds coated with the winged bean seed extract @ 2.5% were found to have the germination and vigour index (63% and 1845), which had no discernible difference from (60% and 1656) 3.5% of the winged bean seed extract.

The increase in germination and vigour index can be attributed to the antioxidant phytochemical present in winged bean seed extract (Table 4). Antioxidant can act at different defence levels such as the first line of defence (preventing free radical formation), the second line of defence (by scavenging them), the third line of defence (by repairing the damages), the fourth line of defence (by generating cell adaptation to the oxidative signals) (Ighodaro *et al.*, 2018). n-Hexadecanoic acid was reported as effective free radical scavengers (Kim *et al.*, 2006). According to Wei *et al.* (2016), oleic acid may be involved in the suppression of oxidative stress by ROS such as nitric oxide (NO) and oxygen anion (O<sub>2</sub><sup>-</sup>). 9,12-Octadecadienoic acid may act as an *in-situ* defensive mechanism against membrane attack by free radicals described by Ha *et al.* (2022).

The result of the bio-assay study showed in Table 7. The adult mortality is significantly higher on cowpea seeds treated with 5.5% ethanol extract of winged bean seeds than the control. However, the extract was most effective with LC<sub>90</sub> value at 2.5%. When compared to other treatments. The death of the insects may be attributed to the presence of compounds like n-hexadecanoic acid, oleic acid, 9,12-Octadecadienoic acid methyl ester, 9,12,15, octadecatrienoic acid as these are the phytochemicals which possess insecticidal properties that kills insects through various mechanisms. These results were in corroboration with Babarinde *et al.* (2016) where

**Table 3:** The bioactive compounds from the hexane seed extract of winged bean by GC-MS analysis.

Retention time	Compounds	Probability	Area (%)
3.1	p-Xylene	36.6	0.52
3.9	2-Heptenal,(Z)-	67.0	0.74
4.2	1-Octen-3-ol	42.2	0.66
4.5	Mesitylene	23.7	0.37
8.4	2-Decenal,(Z)-	51.0	2.65
9.2	2,4-Decadienal,(E,E)-	66.1	1.53
12.4	2,4-Di-tert-butylphenol	51.6	0.33
21.1	Hexadecanoic acid, methyl ester	71.2	0.27
21.6	Dibutyl phthalate	24.5	0.27
22.0	n-Hexadecanoic acid	81.2	11.26
24.3	9,12-Octadecadienoic acid (Z,Z)-,methyl ester	19.5	0.63
24.4	9-Octadecenoic acid, methyl ester,(E)-	11.1	0.80
25.5	9-Octadecenoic acid,(E)-	20.2	16.12
25.6	Ethyl Oleate	30.1	0.42
25.8	Octadecanoic acid	75.7	5.16
28.2	Pyrrolidine, 1-(1-oxo-7,10-hexadecadienyl)-	32.3	0.39
28.3	2-Pyrrolidinone, 1-(9-octadecenyl)-	31.6	0.53
28.6	cis-13-Eicosenoic acid	27.4	1.68
28.9	Eicosanoic acid	73.3	0.53
29.3	Hexadecanoic acid, ethyl ester	19.1	0.29

**Table 4:** Activity of bioactive compounds identified in the different extract of winged bean seeds.

Compound	Nature of compound	Aqueous extract	Ethanol extract	Hexane extract	Property/Activity	Reference
n- Hexadecanoic acid	Saturated fatty acid	✓	✓	✓	Antioxidant,pesticide, nematocide, hemolytic, hypocholesterolemic, anti-androgenic and 5-Alpha reductase inhibitor	Tulika and Mala (2017)
Oleic Acid	Fatty acid	✓	✓	-	Antitoxidant, insecticidal and antibacterial activity	Shaimaa <i>et al.</i> (2021)
Diethyl Phthalate	Plasticizer compound	-	✓	-	Antioxidant, antimicrobial, insecticide, antibacterial and antifungal activity	Ling <i>et al.</i> (2021)
Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl) ethyl ester	Amino compound	✓	-	-	Antioxidant and antimicrobiaactivity	Tulika and Mala (2017)
9,12,15-Octadecatrienoic acid, 2-phenyl-1,3-dioxan-5-yl ester	Fatty acid ester	-	✓	-	Antioxidant, antimicrobial, insectifuge, nematocide and antibacterial activity	Salisu <i>et al.</i> (2019)
2-Decenal(Z)-	Saturated fatty aldehyde	-	-	ü	Antioxidant and nematocidal activity	Shaimaa <i>et al.</i> (2021)
9,12-Octadecadienoic acid (Z,Z)-	Polyunsaturated fatty acid	-	✓	-	Antioxidant, insecticidal, larvicidal activity, nematocide, hypocholesterolemic, hepatoprotective and 5-Alpha reductase inhibitor	Shaimaa <i>et al.</i> (2021)
9,12-Octadecadienoic acid (Z,Z)-,2-hydroxy-1-(hydroxymethyl)ethyl ester	Polyunsaturated fatty acid	✓	-	-	Insectifuge, nematocide, hepatoprotective, hypocholesterolemic and 5-Alpha reductase inhibitor	Sushma <i>et al.</i> (2016)
9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	Fatty acid ester	✓	-	-	Insectifuge activity	Adeniyi <i>et al.</i> (2019)
cis-13-Eicosenoic acid	Fatty acid	-	-	✓	Antifungal activity	Antifungal activity
Vasudha <i>et al.</i> (2021)	Sulfur compound	-	✓	-	Antimicrobial activity	Alagammal <i>et al.</i> (2012)
d-Mannitol,1,4-anhydro-Octadecanoic acid	Saturated fatty acid	-	✓	-	Antimicrobial, antibacterial and antifungal activity	Ibisan <i>et al.</i> (2021)
2,4-Decadienal,(E,E)-	Unsaturated aldehyde	-	-	✓	Nematocidal activity	Pierluigi <i>et al.</i> (2012)
9-Octadecenoic acid,(E)-	Fatty acid	-	-	✓	Acidifier, acidulant, arachidonic acid-inhibitor	Adeniyi <i>et al.</i> (2019)



**Table 5:** Impact of ethanolic winged bean seed extract seed coating on germination (%) of cowpea var. VBN 3 under accelerated ageing.

Treatments (T) Winged bean seed extract	Accelerated ageing duration in days (D)						
	Initial	Day 2	Day 4	Day 6	Day 8	Day 10	Mean
Control (T <sub>0</sub> )	88 (69.73)	84 (66.42)	75 (60.00)	63 (52.53)	54 (47.29)	42 (40.39)	68 (55.55)
0.5% (T <sub>1</sub> )	90 (71.56)	87 (68.86)	78 (62.02)	68 (55.55)	60 (50.76)	48 (43.85)	72 (58.05)
1.5% (T <sub>2</sub> )	92 (73.57)	88 (69.73)	84 (66.42)	72 (58.05)	65 (53.73)	55 (47.87)	76 (60.66)
2.5% (T <sub>3</sub> )	94 (75.82)	91 (72.54)	87 (68.86)	77 (61.34)	70 (56.79)	63 (52.53)	80 (63.43)
3.5% (T <sub>4</sub> )	93 (74.66)	90 (71.56)	85 (67.21)	75 (60.00)	68 (55.55)	60 (50.76)	79 (62.72)
4.5% (T <sub>5</sub> )	91 (72.54)	87 (68.86)	80 (63.43)	72 (58.05)	65 (53.73)	58 (49.60)	76 (60.66)
5.5% (T <sub>6</sub> )	89 (70.63)	85 (67.21)	76 (60.66)	65 (53.73)	55 (47.87)	45 (42.13)	69 (56.16)
Mean	91 (72.54)	87 (68.86)	81 (64.15)	70 (56.79)	62 (51.94)	53 (46.72)	74 (59.34)
	Treatment (T)		Duration (D)			T×D	
SEd	0.62		0.57			1.52	
CD (P=0.05)	1.24		1.14			3.03	

(Figures in parenthesis indicate arc sine values).

**Table 6:** Ethanolic winged bean seed extract seed coating impact on vigour index-I of cowpea var. VBN 3 under accelerated ageing.

Treatments (T) Winged bean seed extract	Accelerated ageing duration in days (D)						
	Initial	Day 2	Day 4	Day 6	Day 8	Day 10	Mean
Control (T <sub>0</sub> )	3572	3166	2670	2022	1495	953	2313
0.5% (T <sub>1</sub> )	3843	3462	2901	2284	1776	1166	2572
1.5% (T <sub>2</sub> )	4140	3722	3276	2512	2041	1424	2853
2.5% (T <sub>3</sub> )	4436	4031	3532	2841	2359	1845	3174
3.5% (T <sub>4</sub> )	4315	3924	3374	2707	2325	1656	3050
4.5% (T <sub>5</sub> )	3994	3532	3016	2491	1976	1484	2749
5.5% (T <sub>6</sub> )	3657	3238	2705	2112	1556	1093	2394
Mean	3994	3582	3068	2424	1933	1374	2729
	Treatment (T)		Duration (D)			T×D	
SEd	26.8		24.8			65.8	
CD (P=0.05)	53.4		49.5			130.9	

**Table 7:** Evaluating the effect of ethanolic winged bean seed extract on adult mortality against *Callosobruchus maculatus*.

Treatments (T) Winged bean seed extract	Hours after treatment (adult mortality%)		
	24 HAT	48 HAT	72 HAT
Control (T <sub>0</sub> )	0.00 (2.87)	0.00 (2.87)	0.00 (2.87)
0.5% (T <sub>1</sub> )	22.75 (28.49)	47.55 (43.60)	69.25 (56.33)
1.5% (T <sub>2</sub> )	30.25 (33.37)	52.75 (46.58)	82.90 (65.61)
2.5% (T <sub>3</sub> )	33.50 (35.37)	72.00 (58.06)	90.50 (72.14)
3.5% (T <sub>4</sub> )	35.50 (36.57)	69.75 (56.65)	92.75 (74.69)
4.5% (T <sub>5</sub> )	40.55 (39.55)	75.50 (60.35)	95.25 (77.83)
5.5% (T <sub>6</sub> )	42.00 (40.40)	78.75 (62.57)	97.75 (82.43)
Mean	29.22 (32.72)	56.61 (48.80)	75.49 (60.32)
SEd	0.45	0.94	2.54
CD (P=0.05)	0.98	2.03	5.46

(Figures in parenthesis indicate arc sine values).

they reported the insecticidal properties of these mentioned compounds. Furthermore, Dobie *et al.* (1979) reported that mature winged bean seeds seem to be resistant to bruchid infestation.

## CONCLUSION

The present study concluded the presence of many phytochemicals in winged bean seed extracts of which, the ethanolic seed extract showed the potent bioactive compounds, which are valuable in seed storage, compared with aqueous and hexane extract. The ethanolic seed extract treatment maintained the vigour and viability of cowpea seeds under accelerated ageing when compared to untreated seeds. It was also found to be effective at controlling pulse beetle. This is environmentally safe to treat seeds compared to conventional pesticides. However, GC-MS is the first step towards understanding the nature of active principles present in winged bean seeds more research on isolating and identifying individual component and subjecting it to a biological activity and toxicity profile will undoubtedly provide fruitful results.

**Conflict of interest:** None.

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