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In silico and *In vitro* Tests of Phytocompounds Extracted from Leaves of *Plectranthus amboinicus (Lour.)* Spreng as Biopesticides Against Enzymes, Proteins and Selected Cell Lines of the Coleoptera *Callosobruchus maculatus (Fabr., 1775)*

C. Shunmugadevi [a++*], S. Anbu Radhika [b#] and P. Palanisamy [c]

[a] PG and Research Department of Zoology, PMT College, India.
[b] PG and Research Department of Zoology, PMT College, Melaneelithanallur-627637, India.
[c] Research Department of Chemistry, Pioneer Kumaraswamy College, Nagercoil-629003, Affiliated to Manonmananiam Sundaranar University, Tirunelveli-627012, Tamil Nadu, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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[++] *Research Scholar;*
[#] *Assistant Professor;*
**Corresponding author: Email: ppsdevi2018@gmail.com;*

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ABSTRACT

The majority of synthetic pesticides have negative consequences on both the environment and human health, making insect pest management a global economic and ecological disaster. Crude extracts of phytocompounds from *Plectranthus amboinicus* were tested for their pesticidal effects on a certain enzyme cell line. An *in silico* molecular docking analysis of phytocompounds showed that the enzymes Glutathione S-Transferase (7RKA), Mytocontrial (5H3F), Acetylcholinesterase (7QAK), GABA receptor (7WGT), and DNA nucleotidylexotransferase (6GO4) interacted strongly with the phytocompounds. Usnic acid showed higher in-silico biopesticidal activity against Callosobruchus maculatus when compared to reference pesticide Dichlorvos and Malathion. A silica gel column chromatographic technique with appropriate solvent extract has been used to isolate the highly active components of usnic acid. *In vitro* studies revealed that, after 96 hours, The Usnic acid mean observed mortality percentage is 49.6% (24hr), 64.8 (48hr), 74.8% (72hr) and 87.2% (96hr). causes the maximum toxicity of *Callosobruchus maculatus* compared to the reference pesticides Diclorvos (84.6% & LC50 value 6.65 mg/ml) and Malathion (92% & LC50 value 5.62 mg/ml). Usnic acid exhibits promise as a pesticidal agent when compared to the reference medications. Consequently, these specific chemicals might offer substitute therapies that augment the traditional applications of the plants that are being studied.

Keywords: In vitro and In-silico molecular docking analysis; Plectranthus amboinicus; bio-pesticidal activity; toxicity analysis.

1. INTRODUCTION

The most dangerous pest for grain pulses that are kept in storage is the bruchid, particularly in tropical and subtropical regions. When grains are stored after harvest, they also seep into fully developed, mature pods that are growing in the fields. *Callosobruchus maculatus* is the most common bruchid pest, found all across the world. Chemical and synthetic pesticides are effective in controlling *C. maculatus* on pulses [1]. Most pests are classified as belonging to the orders Lepidoptera (100%) and Coleoptera (60%) [2]. *C. maculatus* is one of the most harmful pulse beetles and a major cowpea pest. It severely damages most pulses. This beetle was taxonomically classified by Kergoat et al. [3] into the subfamily Bruchinae and family Chrysomelidae. Pulses and grains are attacked by *C. maculatus* during post-harvest storage, resulting in substantial loss. Cowpea, which *C. maculatus* dominates, is one of the greatest examples [4].

Secondary metabolites found in plants, such as phenolics, terpenoids, and alkaloids, have the ability to interfere with plant-eating insects' sense organs, preventing them from laying eggs and feeding [5]. According to Huang et al. [6], nutmeg (M. myristica) oil contains antifeedant properties that it may use to combat *Sitophilous zeamais* and *Tribolium castaneum*. The essential oils of several medicinal plants have been employed to combat insects in stored products [7]. The five plant components, which include major spices like African nutmeg, Manjack, Ginger, Galic, and Negro pepper, were found to be efficient against cowpea weevil by Andy and Edema [8]. T This led to the discovery that C. maculatus feeding is inhibited by ginger extract. Lemon peel powder has more efficiency than orange peel powder when it comes to treating *Callosobruchus macualtus*, according to research carried out by Fawkes et al. (2014). Arora et al. [9] discovered a number of obstacles in the way of giving small-scale farmers access to these botanical extracts. For instance, plant extracts are not readily available for purchase. So, farmers are stuck with chemically synthesized pesticides instead of natural ones. In addition, the primary obstacles are the issues with plant-based pesticide manufacture and delivery. As a result, it's necessary to launch an awareness campaign where locals and farmers are informed about the usage of plant-based pesticides [10].

Plectranthus amboinicus leaves powdered extract showed a higher level of toxic and inhibitory activity against *Callosobruchus maculatus*. Among the various solvent plant

extracts, the petroleum benzene and ethanol were the most effective with high toxic compounds. The stored grain pest may be effectively controlled using conventional plant treatments, therefore mitigating the significant damage caused by insect pests *Callosobruchus Maculatus* [11].

In cholinergic synapses, which are vital for insects and larger animals, acetylcholinesterase (AChE) plays a function [12]. Acetylcholine builds up at synapses when AChE is inhibited, causing the post-synaptic membrane to remain permanently stimulated. This leads to ataxia, or a general loss of coordination in the neuromuscular system, and may even cause death [13]. Insecticides that function as antagonists by stabilizing non-conducting conformations of the chloride channel have been shown to bind to ligand-gated chloride channels. Neuronal inhibition is decreased when the Gamma-aminobutyric acid (GABA) -gated chloride channel is blocked, which causes the central nervous system to become hyperexcited and cause convulsions and even death [14]. Herbivorous insects have been shown to respond to GABA and related aminobutyric acids by stimulating feeding and evoking taste receptors; these allelochemicals, however, oppose GABA phagostimulants and cause feeding deterrent [15]. Additionally, insecticidal poisoning can happen via interfering with the action of an enzyme system (like ATPase) or protein phosphorylation (H-ATP: proton pump) (Cheng and Fu, 1989). A well-known and powerful mitochondrial toxin, rotenone works by preventing mitochondrial function [16]. In insects, octopamine (OA) is the focus of essential oil action. Ozone is a naturally occurring, multipurpose biogenic amine that functions physiologically similarly to nornephrine in vertebrates. It serves important roles as a neurotransmitter, neuromodulator, and neurohormone in the invertebrate system [17]. Based on the previously stated information, we may continue to study the in vitro and in silico biopesticidal activity of phytocompounds extracted from the Plectranthus amboinicusplant against certain enzymes, proteins, cell lanes, and Callosobruchus maculatus.

2. MATERIALS METHODS

2.1 Collection and Authentication of Plant Materials

We gathered leaves from *Plectranthus amboinicus* in Kasilingapuram village, Thoothukudi district, Tamilnadu, India (8°46'30.4"N 77°53'20.2"E) in October and November of 2021. The plant specimens were identified and verified by Dr. C. Babu, Head and Associate Professor of Botany at Pioneer Kumaraswamy College in Nagercoil. After giving the leaves a thorough cleaning under running water, they were allowed to air dry for seven or eight days at room temperature in the shade. After being ground into a fine powder, plant leaves are stored for later use in an airtight container.

2.2 Extraction of Plant Materials

A Soxhlet extractor was used to dissolve 50 grams of dry leaves in 250 ml of petroleum benzine (40–60°C), benzene, chloroform, ethanol, and water. The extraction process involves adding solvents to the Soxhlet loop until the solvent is colorless [18]. As the solvent evaporated, the concentrated extracts were stored in sealed containers at room temperature. After that, the mixture was frozen at 4°C for later use [19].

2.3 Molecular Docking

Using the conventional approach, AutoDock Vina was utilized to dock the proteins and isolate the chemical component into the protein's active site. Using the ChemOffice program (Chem Draw 16.0) and the appropriate 2D orientation, the chemical structures of the compounds were drawn, and ChemBio3D was used to reduce the energy of each molecule. To perform the docking simulation, AutoDock Vina received the 3D structures as input. Protein Data Bank was used to download the crystal structures of the following receptor molecules: DNA nucleotidylexotransferase enzyme (PDB ID: 6GO4), acetylcholinesterase enzyme protein (7QAK), GABA receptor enzyme protein (PDB ID: 7WGT), and glutathione S-Transferase enzyme protein (PDB ID: 7RKA). The target protein file was prepared by leaving the associated residue with protein using Auto Dock 4.2 (MGL tools1.5.7), which automates the preparation of target protein files. The protein preparation was carried out according to the reported standard protocol, which involved removing the co-crystallized ligand, selected water molecules, and cofactors. The grid box for the docking simulations was configured using the graphical user interface software. With a grid point spacing of 0.375 Å, the grid box was built with 40, 40, and 40 pointing in the x, y, and z

directions, respectively. The coordinates of the center grid box for 7RKA, 5H3F, 7QAK, 7WGT, and 6GO4 are (52.98Å, 7.75Å, and 9.22Å), (-76.47Å 27.00 Å, and 48.58 Å), (17.92Å, 10.68 Å, and -6.26 Å), (1.38 Å, -5.18 Å, and 36.54 Å), and (-13.69 Å, 11.12 Å, and 21.01 Å), respectively. The docking approach offered with Auto Dock Vina was used to find the best possible docked arrangement between the protein and the ligand. For every ligand, a maximum of nine conformers were taken into consideration throughout the docking procedure. Using PyMOL and Discovery Studio Visualizer, the conformations with the most advantageous (least) free binding energy were chosen to examine how the ligands interacted with the target receptor. The stick model depiction of the ligands is colored differently from that of the H-bonds and interacting residues [20].

2.4 Insect Collection and Rearing

In the village of Kasilingapuram, Thoothukudi District, Tamilnadu, India, a farmer provided the Callosobruchus maculates (Fab.) insects for collection. In the PG and Research Department of Zoology at PMT College, Melaneelithanallur, Tenkasi District, Tamilnadu, India, collected insects were kept in laboratory conditions. 77°53'20.2"E, 8°46'30.4"N. Initially, fifty adult pairs that had been together for no more than two days were put in jars with cowpea seeds and given a maximum of seven days to mate and produce eggs. Utilizing cowpea grains, pests were raised at 28 ± 1oC and 65 ± 5% relative humidity (RH). To avoid contamination and insect escape, the eggs from the cowpea seeds were taken out of their parents and placed in new jars with rubber bands and fabric covers to protect the seeds from contamination. In every experiment we conducted, we employed pest progenies [21].

2.5 Boipesticital Activity

Plectranthus amboinicus solvent extracts were tested for toxicity against mature C. maculates at 28 ± 1oC and 65 ± 5% relative humidity. Newly emerging adults (aged 1–15 days) were employed in these investigations. In glass jars (replicas) with filter paper (3 x 3 cm) fastened to screw caps on the underside, extracts from different plants were evaluated for their capacity to kill mature C. maculates. Plant extracts were administered to 30 flies in each jar at doses of 10, 20, 30, 40, and 50 mg/ml. For every treatment and control, the process was done five times. Water and only the solvent were utilized as a control. The chi-square, lethal concentration causing 50% mortality (LC50) was determined after 24, 48, 72, and 96 hours for each concentration, based on log-concentration mortality regression lines. The insects were regarded as dead as long as their legs or antennae were still and seemed paralyzed [22].

2.6 Statistical Analysis

In order to determine whether there were significant differences between the variable concentrations ($P < 0.05$), we performed an analysis of variance (ANOVA) and a least significant difference (LSD) multiple range test on the data. When their respective 95% fiducial limits did not overlap for pesticidal and repellent activity, that was taken into consideration [23].

3. RESULTS AND DISCUSSION

3.1 Phytocompounds Identification

We investigated the phytochemistry of plant extracts from *Plectranthus amboinicus* GCMS analysis using the SHIMADZU (QP2020) GC-MS equipment. With additional patterns, the data from WILEY (Hubschmann, 2015) and NIST (Stephen, 2012) were used to evaluate mass spectra and gas chromatograms. Using the NIST and WILEY libraries, we compared the spectra of the unknown and known components. Through GC-MS, it is possible to identify the functional groups that comprise the bioactive components of terpenoids, steroids, fatty acids, phenolic compounds, alkaloids, saponins, and flavonoids. In this study, we investigate the Gas Chromatography-Mass Spectroscopy findings on the different solvent extracts of *Plectranthus amboinicus*, as displayed in Tables 1 and 2. Nine of the twenty-five chemicals found in the petroleum benzine extract tested hazardous in nature. Thymol (43.03), beta caryophyllene (3.25), farnesol (1.4), octadecanal (1.2), phytol (0.71), codlelure (0.5), (Z)-11-hexadecenal (1.36), erucic acid (0.71), and squalene (17.45) were hazardous chemicals found in the petroleum benzine extract of *P amboinicus*. Twenty different chemicals were found in benzene extracts, and five of them seemed hazardous. Carvacrol (43.03), Palmitic acid (1.11), Oleic acid (1.36), Dioctyl phthalate (6.65), and beta-Pregnane (2.96) are some of the hazardous substances found in benzene extracts. Five of the 17 substances found in the chloroform extracts that were identified were

poisonous substance like phytol (1.92), palmitic acid (11.76), decahydronaphthalene (1.12), (Z)-11-hexadecenal (7.96), and dioctyl phthalate (44.86). Twenty chemicals were found in the ethanol extracts, eight of which were hazardous, such as palmitic acid (7.84), 1-dodecanethiol (1.98), 1-dodecene (4.26), 2,5-di-tert-amylhydroquinone (8.15), hexacosanoic acid (6.53), genipin (2.36), and propylparaben (2.33). The water extracts revealed the presence of 10 different chemicals, two of which were hazardous, including 2,4-di-tert-butylthiophenol (7.17) and usnic acid (10.24). Here also the Petroleum benzine extracts retrieved 25 compounds than other solvent extracts of *P. ambonicus*. According to Mohammed et al. [24] lauric acid is a 12-carbon medium-chain saturated fatty acid that has both a chemical and physical insecticide effect. [25].

3.2 Molecular Docking Analysis

In the different solvent extracts from *Plectranthus amboinicus*, 28 toxicadal phytocompounds were identified. In comparison to the reference drugs, all 28 phytocompounds exhibited comparable toxicity activity. Glutathione S-Transferase enzyme Protein (7RKA) molecular docking binding energy score ranges from -3.5 to -7.5 kcal/mol, Mytocontrial enzyme Protein (5H3F) from -3.9 to -5.9 kcal/mol, Acetylcholinesterase enzyme protein (7QAK) from -5.4 to -8.6 kcal/mol, GABA receptor enzyme protein (7WGT) from -3.9 to -9.9 kcal/mol and DNA nucleotidylexotransferase enzyme (6GO4) -4.5 to -7.5 kcal/mol. The ethanol extract oxandrolone phytocompounds have more antibacterial activity than reference drugs. The phytocompound Cholecalciferol water-based extracts exhibits superior toxicity activity to the reference drugs against Glutathione S-Transferase enzyme Protein (7RKA), Mytocontrial enzyme Protein (5H3F), Acetylcholinesterase enzyme protein (7QAK), GABA receptor enzyme protein (7WGT), and DNA nucleotidylexotransferase enzyme (6GO4).

Therefore, the ethanol extract of *Plectranthus amboinicus* (5 g) was subjected to silica gel flash column chromatography and eluted with an increasing gradient of ethyl acetate in *n*-hexane. A total of 15 fractions were collected (each 25mL). Fraction 9-12 were combined and subjected to silica gel column chromatography and eluted with an increasing gradient of ethyl acetate in *n*-hexane. A total of 2 fractions (15 mL each) were collected. Sub fraction 2 eluted with 40% ethyl acetate in n-hexane afforded Usnic acid (7, 600 mg) [26,27].

Usnic acid (**7**) as a colourless solid (600 mg); mp 202-204 ^{0}C; FT-IR (KBr): 1668 (-C-C=O), 1720 (-CH_3-C=O), 2909 (-CH3) and 3444(-OH) cm^{-1}. ^{1}H-NMR (400MHz, CDCl3) 1.39 (-CH3), 1.90 and 2.31 (-CO-CH_3), 2.64 (-CH_3), 4.03 (-CH), 5.02 (bs, -OH), and 5.83 (-C=CH). ^{13}C-NMR (400MHz, DCl3) □ ppm 7.65, 20.14, 28.53, 34.52, 50.06, 99.12, 104.65, 110.63, 113.26, 155.63, 160.32, 163.51, 180.52, 183.64, 194.62, 198.63 and 202.36(C=O); HRMS m/z: 344.32 (MF $C_{18}H_{16}O_7$).

3.3 Molecular Docking Analysis of Isolated Phytocompounds

Usnic acid showed a strong affinity for Glutathione S-Transferase enzyme Protein (7RKA), Mytocontrial enzyme Protein (5H3F), Acetylcholinesterase enzyme protein (7QAK), GABA receptor enzyme protein (7WGT), and DNA nucleotidylexotransferase enzyme (6GO4), with binding energies of -7.5, -5.9, -7.6, -8.7

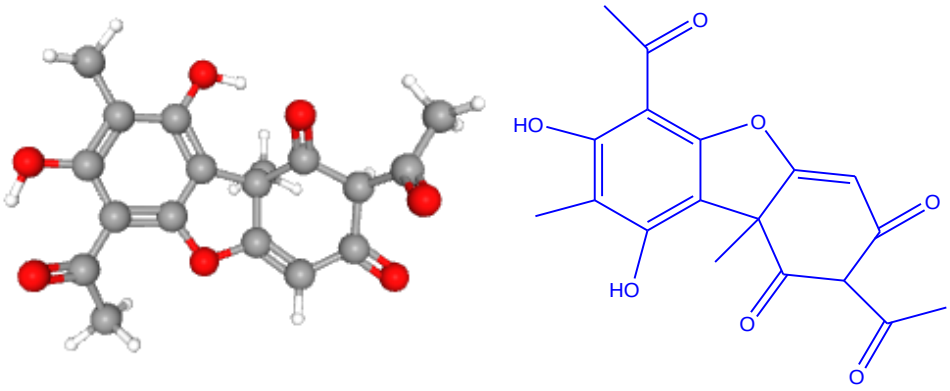


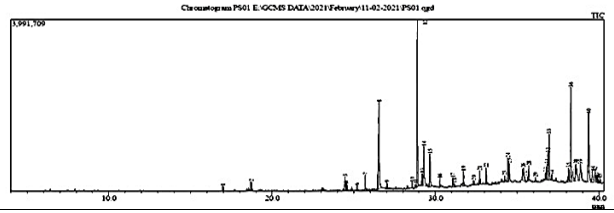
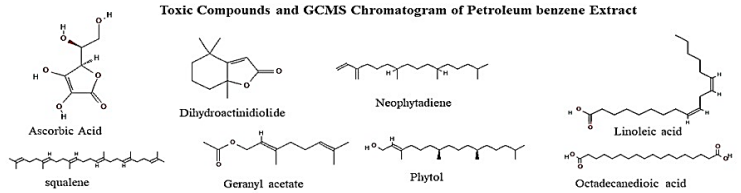
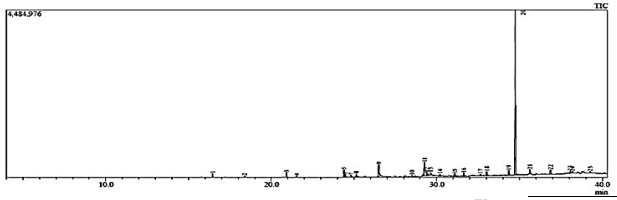
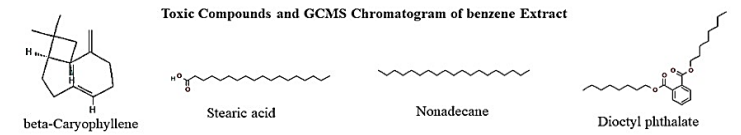
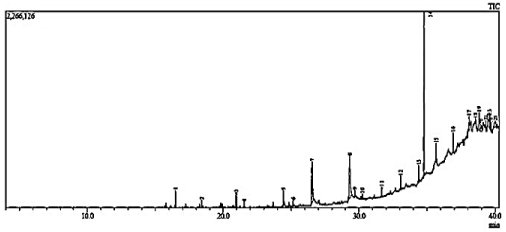
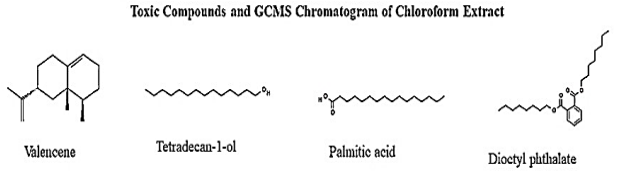
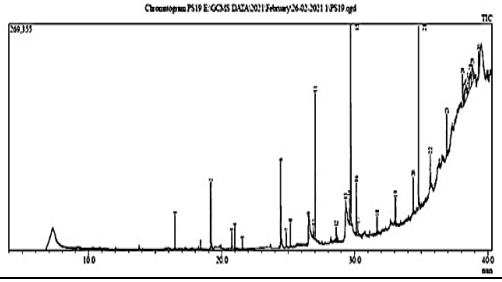
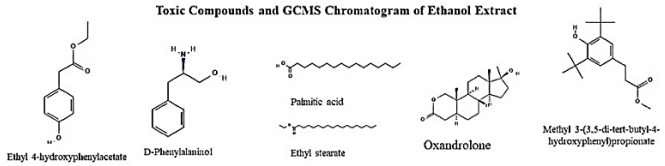
Fig. 1. Structure of Usnic acid (7)

Table 1. GC-MS analysis of phytocompounds from *Plectranthus amboinicus* leaves different solvent extract

Solvent	Retention Time (min)	Peak Area	m/z value	Molecular formula	Name of the Compound	Name of the Phytochemical	Toxicity
Petroleum benzine	16.984	0.39	194	C12H20O2	Geranyl acetate	monoterpenoid	Skin, Eye, and Respiratory Irritations Health Hazards
	18.707	0.79	180	C11H16O2	Dihydroactinidiolide	Lactone	Acute toxicity, oral
	24.454	1.21	278	C20H38	Neophytadiene	diterpene	Health Hazards
	25.188	1.59	296	C20H40O	Phytol	diterpenoid	Skin, Eye, and Respiratory Irritations Health Hazards
	26.544	6.36	652	C38H68O8	Ascorbic Acid	Vitamins	Fungicides
	29.183	1.31	280	C18H32O2	Linoleic acid	omega-6 fatty acid	Skin, Eye, and Respiratory Irritations
	29.658	3.56	284	C18H34O4	Octadecanedioic acid	alpha,omega-dicarboxylic acid	Skin, Eye, and Respiratory Irritations
	38.280	8.48	410	C30H50	squalene	triterpene	Health Hazards
Benzene	16.453	1.16	204	C15H24	beta-Caryophyllene	sesquiterpene	Skin, Eye, and Respiratory Irritations Health Hazards
	29.630	1.42	284	C18H36O2	Stearic acid	long-chain fatty acid	Skin, Eye, and Respiratory Irritations
	30.195	0.61	268	C19H40	Nonadecane	Hydrocarbon	Skin, Eye, and Respiratory Irritations Health Hazards
	37.750	58.18	390	C24H38O4	Dioctyl phthalate	Phthalic Acids	Skin, Eye, and Respiratory Irritations Health Hazards
Chloroform	18.418	1.18	204	C15H24	Valencene	Sesquiterpenes	Skin, Eye, and Respiratory Irritations Health Hazards
	21.555	0.82	214	C14H30O	Tetradecan-1-ol	Fatty Alcohols	Health Hazards
	26.539	10.43	256	C16H32O2	Palmitic acid	Fatty acids	Skin, Eye, and Respiratory Irritations

Solvent	Retention Time (min)	Peak Area	m/z value	Molecular formula	Name of the Compound	Name of the Phytochemical	Toxicity
	37.770	25.54	390	C24H38O4	Dioctyl phthalate	Phthalic Acids	Health Hazards Skin, Eye, and Respiratory Irritations
Ethanol	19.168	5.6	180	C10H12O3	Ethyl 4-hydroxyphenylacetate	ester	Health Hazards Skin, Eye, and Respiratory Irritations
	20.744	1.67	151	C9H13NO	D-Phenylalaninol	aminoacids	Health Hazards
	26.532	3.69	256	C16H32O2	Palmitic acid	Fatty acids	Health Hazards Skin, Eye, and Respiratory Irritations
	30.115	3.25	312	C20H40O2	Ethyl stearate	Fatty aster	Health Hazards Skin, Eye, and Respiratory Irritations
	38.560	1.24	306	C19H30O3	Oxandrolone	oxa-steroid	Skin, Eye, and Respiratory Irritations Health Hazards
	38.560	3.09	306	C18H28O3	Methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate	Ester	Skin, Eye, and Respiratory Irritations Health Hazards
Water	30.640	1.67	454	C27H44O	Cholecalciferol	steroid	Rodenticides, Acute toxicity, oral
	35.388	1.28	262	C18H30O	4-Dodecylphenol	Phenolic compounds	Skin, Eye, and Respiratory Irritations Health Hazards

Table 2. GC-MS chromatogram of *Plectranthus amboinicus* plant leaves extracts

Solvent	GC- MS Chromatogram	Toxic Phytocompous
Petroleum benzine		
Benzene		
Chloroform		
Ethanol		

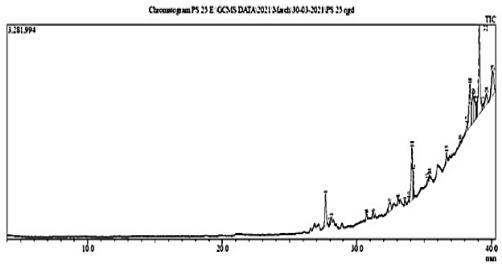
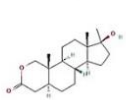
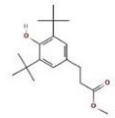
Water		Toxic Compounds and GCMS Chromatogram of Water Extract  Oxandrolone  Methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate

Table 3. Molecular Docking Analysis of *Plectranthus amboinicus* phytocompounds Against Slected enzyme protein cell lane

S. No	Solvents	Phytocompounds	Glutathione S-Transferase enzyme Protein (7RKA)	Mytocontrial enzyme Protein (5H3F)	Acetylcholinesterase enzyme protein (7QAK)	GABA receptor enzyme protein (7WGT)	DNA nucleotidylexotransferase enzyme (6GO4)
1	Petroleum Benzene	Thymol	-5.4	-5.6	-7.8	-6.0	-5.7
2		Beta Caryophyllene	-6.3	-5.1	-6.3	-7.5	-6.3
3		farnesol	-5.1	-5.8	-6.7	-6.7	-4.9
4		Octadecanal	-4.3	-4.4	-6.3	-5.5	-4.1
5		Phytol	-4.8	-5.0	-6.7	-6.3	-5.1
6		Codlelure	-4.7	-5.8	-5.4	-5.7	-4.6
7		(Z)-11-Hexadecenal	-4.5	-4.8	-7.4	-5.5	-4.6
8		Erucic acid	-4.2	-5.9	-7.6	-5.4	-4.4
9		squalene	-5.6	-3.9	-8.6	-7.9	-5.3
10	Benzene	Carvacrol	-5.5	-5.6	-7.6	-6.2	-6.0
11		Palmitic acid	-4.1	-4.6	-6.2	-5.7	-4.7
12		Oleic acid	-4.4	-4.7	-6.4	-6.0	-5.0
13		Dioctyl phthalate	-5.1	-4.2	-6.8	-6.7	-5.0
14		5beta-Pregnane	-7.6	-6.1	-6.6	-9.9	-6.5
15	Chloroform	Phytol	-4.8	-5.0	-6.7	-6.3	-5.1
16		Palmitic acid	-4.1	-4.6	-6.2	-5.7	-4.7
17		Decahydronaphthalene	-4.3	-4.7	-6.4	-6.9	-5.6
18		(Z)-11-Hexadecenal	-4.5	-4.8	-5.4	-4.8	-4.6

S. No	Solvents	Phytocompounds	Glutathione S-Transferase enzyme Protein (7RKA)	Mytocontrial enzyme Protein (5H3F)	Acetylcholinesterase enzyme protein (7QAK)	GABA receptor enzyme protein (7WGT)	DNA nucleotidylexotransferase enzyme (6GO4)
19		Dioctyl phthalate	-5.1	-4.2	-6.8	-6.7	-5.0
20	Ethanol	Decyl acrylate	-4.0	-5.3	-6.6	-5.6	-4.5
21		1-DODECANETHIOL	-3.7	-5.1	-6.5	-5.1	-3.9
22		1-Dodecene	-3.5	-5.8	-6.6	-5.1	-4.5
23		2,5-Di-tert-amylhydroquinone	-6.2	-5.3	-5.6	-7.4	-5.4
24		Hexacosanoic acid	-4.6	-4.6	-6.1	-4.0	-4.7
25		Genipin	-5.1	-6.2	-7.3	-6.6	-5.8
26		Propylparaben	-5.2	-5.0	-7.6	-5.9	-5.6
27	Water	2,4-Di-tert-butylthiophenol	-6.0	-5.2	-5.9	-6.8	-4.9
28		Usnic acid	-7.5	-5.9	-7.6	-8.7	-7.5
29	Standards	Dichlorvos	-3.9	-4.1	-5.7	-4.7	-4.3
29		Malathion	-4.4	-4.2	-4.2	-5.3	-4.4

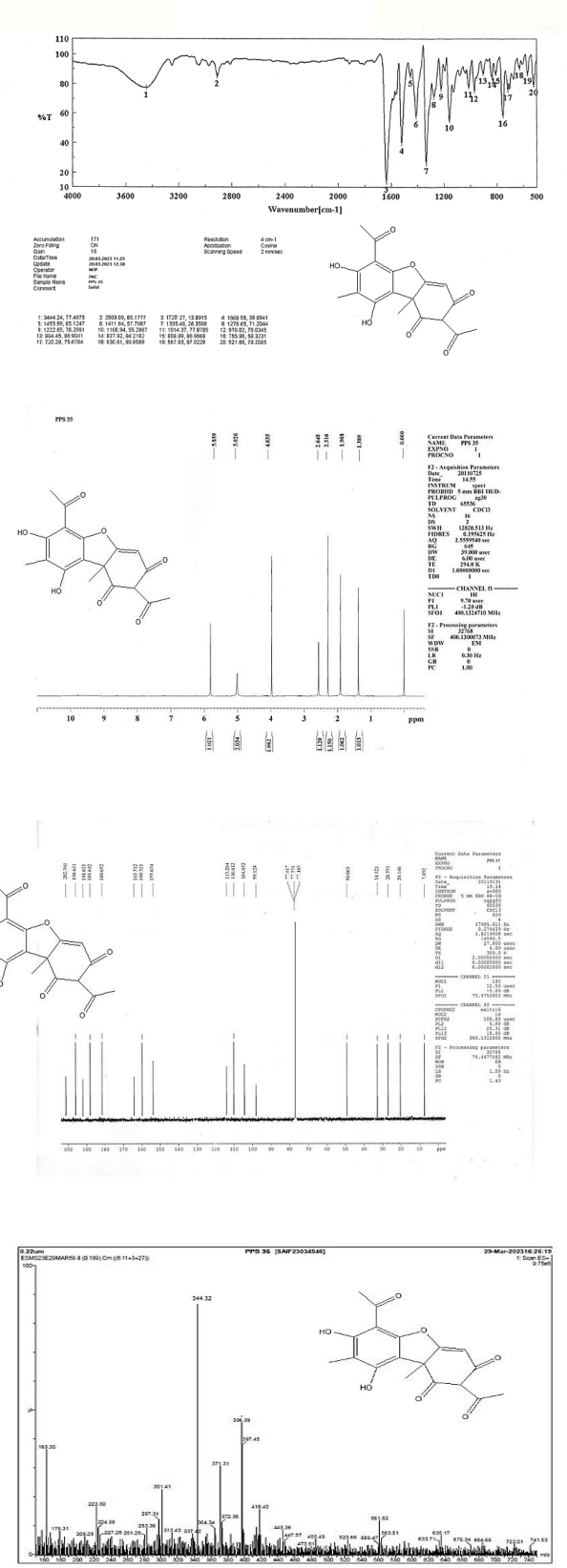


Fig. 2. IR, 1H-NMR, 13C-NMR and Mass Spectra of Usnic acid (7)

and -7.5 kcal/mol in comparison to the standard reference drugs Dichlorvos (-3.9, -4.1, -5.7, -4.7 and -4.3 kcal/mol) and malathion (-4.4, -4.2, -4.2, -5.3 and -4.4 kcal/mol) (Table 2 and Fig. 3). The glutathione S-Transferase enzyme Protein (7RKA) interacted with the Usnic acid molecule through 12 hydrogen bonds, including VAL8, GLY11, TYR107, TYR107, TYR107, SER111, GLU112, LEU115, ALA116, PHE119, PHE119, and SER14, five hydrophobic bonds CYS169, PHE119, TYR107, TYR107 and TYR107. The Usnic acid molecule interacts with the Mytocontrial enzyme Protein (5H3F) cell lane protein, which has 6 hydrogen bonds namely LYS133, LYS180, LYS180, LYS256, LYS256, LYS256, LYS256, ALA257, ALA257, GLY260, and ARG261, seven hydrophobic bond such as ARG149, LYS251, ARG140, ARG140, ARG172, ARG172, and MG502. Usnic acid displayed 23 hydrogen bonds (LYS332, ASP333, SER336, LEU339, TYR382, ASP396, ALA400, ASP404, VAL408, CYS409, ALA412, PHE430, VAL445, HIS447, TYR449, TYR510:, GLY523, ARG525, THR528, CYS529, PHE531, TRP532 and ASN533), seventeen hydrophobic bonds (HIS381, VAL401, HIS447, ASP404, HIS405, ASP404, HIS405, VAL330, VAL331, LYS332, ALA412, ARG433, PRO446, ARG525, ARG525, TYR382 and TYR449) and one electrostatic bonds (GLU202) in its interactions with the Acetylcholinesterase enzyme protein (7QAK), respectively.

The GABA receptor enzyme protein (7WGT) interacted with the Cholecalciferol molecule through 17 hydrogen bonds, including ARG52, PHE53, TYR55, LEU56, LYS59, ASN60, LEU120, TYR123, TYR124, VAL126, ILE127, TRP130, SER131, ASP312, GLN316, GLY385, and LEU388, fifteen hydrophobic bonds VAL389, TYR124, LEU47, ARG52, VAL389, ALA478, :ALA479, TRP51, TYR55, PHE65, TYR123, TYR124, TRP130, PHE470, and PHE471. The Usnic acid molecule interacts with the DNA nucleotidylexotransferase enzyme (6GO4) cell lane protein, which has ten hydrogen bonds namely VAL344, PHE346, GLN379, HIS381, ARG404, PHE406, ARG433, ARG433, ASP435 and NA601, three hydrophobic bond such as ARG404, ARG433 and PHE406 and one electrostatic bonds namely ARG404.

3.4 Biopesticidal Activity

3.4.1 Biopesticidal activity of isolated phytocompounds

The isolated phytocompounds showed excellent pesticidal activity against *C. maculatus*, and the time and dose needed to cause 50% (LC50) mortality dropped with increased concentration. Plant extracts from the leaves were tested against the pest with various concentrations of 10, 20, 30, 40, and 50 mg/ml and exposure times of 24, 48, 72 and 96 hours, respectively. The Usnic acid (1) mean observed mortality

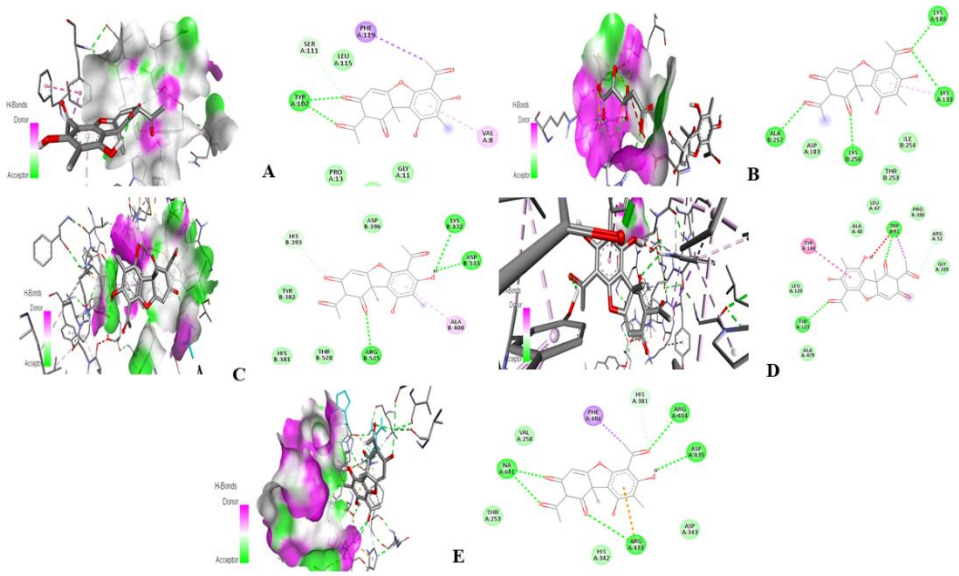


Fig. 3. Molecular docking analysis of Usnic acid (7) phytocompounds against Glutathione S-Transferase enzyme Protein (A), Mytocontrial enzyme Protein (B), Acetylcholinesterase enzyme protein (C), GABA receptor enzyme protein (D), and DNA nucleotidylexotransferase enzyme (E)

Table. 4. Toxicity analysis of isolated *phytocompounds* againnt *Callosobruchus maculatus*

Plant Material	Concentration (mg/ml)									LC_{50}	$\chi 2$
	Exposure Times (Hours)	10	20	30	40	50	Mean	P value	Significant		
Usnic acid	24	32	36	48	60	72	49.6	0.02	<0.05	26.59	1.543
	48	44	52	64	80	84	64.8	0.001	<0.05	14.76	1.411
	72	50	68	76	88	92	74.8	0.003	<0.05	8.06	1.235
	96	72	80	88	96	100	87.2	0.001	<0.05	5.95	2.207
Dichlorvos	24	20	28	36	56	68	41.6	0.000	<0.05	35.00	1.936
	48	36	60	64	72	80	62.4	0.001	<0.05	16.07	0.329
	72	60	72	80	84	92	77.6	0.004	<0.05	7.24	0.450
	96	72	80	88	92	100	86.4	0.001	<0.05	5.94	2.207
Malathion	24	44	52	60	68	76	60	0.013	<0.05	15.52	0.451
	48	56	64	72	80	88	72	0.007	<0.05	8.96	0.786
	72	68	76	84	92	100	84	0.001	<0.05	6.66	2.685
	96	76	84	92	100	100	92	0.001	<0.05	5.62	2.645

percentage is 49.6% (24hr), 64.8 (48hr), 74.8% (72hr) and 87.2% (96hr). The reference pesticide Dichlorvos mean observed mortality percentage is 41.6 (24hr), 62.4 (48hr), 77.6 (72hr) and 86.4 (96 hr) and mean mortality activity of Malathion observed 60, 72, 84, and 92% in 24, 48, 72, and 96 hours respectively. From the above results the Usnic acid phytocompounds showed highest pesticidal activity than the other phytocompounds in *Plectranthus amboinicus* plant extract.

4. CONCLUSION

According to this study, *Plectranthus amboinicus* phytocompounds have in silico pesticidal efficacy against certain enzymes and cell lane proteins. The compounds with isolated pesticidal efficacy against Callosobruchus maculatus were tested in vitro and in silico. Compounds Lupeol (1) showed high interaction with *Glutathione S-Transferase enzyme protein (7RKA), Mytocontrial enzyme protein (5H3F), Acetylcholinesterase enzyme protein (7QAK),* and *GABA receptor enzyme protein (7WGT) in silico* molecular docking studies. Compared to Dichlorvos and Malathion, the chemicals guanosine shown higher in vitro biopesticidal action against Callosobruchus maculatus. After 96 hours, *Plectranthus amboinicus* (87.2% & LC50 value 5.95 mg/ml) induced the maximum toxicity in Callosobruchus maculatus, compared to reference pesticides Dichlorvos (84.6% & LC50 value 5.94 mg/ml) and Malathion (92% & LC50 value 5.62 mg/ml). Compared to other native plants, guanosine is more accessible to farmers because to its affordability and ease of adaptation, as well as its shown efficiency and effectiveness in preserving cowpea seeds. To find out the product's active ingredient, cost-benefit analysis, and capacity to manage infestations in grain warehouses, more research is also necessary.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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