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Exploration of Anticancer, Antioxidant, Antibacterial Potential of Eulophia Guineensis and Identification of its Bioactive Phytoconstituents

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KEYWORDS

Eulophia guineensis, anticancer, MTT, DPPH, antioxidant, antibacterial

ABSTRACT:

Introduction: Eulophia guineensis (Orchidaceae) is a small perennial terrestrial herb found in central and Southeast Asian.

Objectives: To evaluate the anticancer, antioxidant and antibacterial potential of Eulophia guineensis (Orchidaceae) in vitro.

Methods: The anticancer and antioxidant property was determined by MTT assay and DPPH (2,2 Diphenyl-1- Picrylhydrazyl) assays respectively. The presence of active phytoconstituents was determined by UV and IR techniques. The MTT assay was performed usinvg MCF-7 human breast cancer cell line. Antibacterial activity was determined by well diffusion method. Well diffusion method was used against Escherichia coli and Staphylococcus aureus.

Results: The Eulophia guineensis extract (EGE) at 1000 ($p<0.001$), 250 ($P<0.01$),125($P<0.05$) µg/ml concentration showed significant decrease in cell viability in comparison to control. It showed IC50 value 417.30±10.71µg/ml. It DPPH radical scavenging activity was observed in a concentration dependent (50-500µg/ml) manner. It showed IC50 value 417.30±10.71µg/ml with the correlation coefficient of 0.9967. The methanol extract showed antibacterial activity against S. aureus with maximum zone of inhibition of 10 mm.

Conclusion: The study confirmed, anticancer activity, antioxidant activity and antibacterial activity of Eulophia guineensis.

1. Introduction

The most common cancer in women is breast cancer. While the majority of breast cancers are benign and treatable with surgery, 25% have a latent, sneaky nature that causes them to grow slowly but spread quickly [1, 2]. Reactive oxygen species (ROS) have been linked to a number of degenerative disorders in humans, such as cancer, aging, and neurological conditions like Alzheimer's, Parkinson's, and Huntington's diseases [3]. When it was first believed that oxidative stress influenced apoptosis, antioxidants have been utilized to suppress apoptosis [4]. The first line of defence against oxidative stress and damage from free radicals is comprised of antioxidant enzymes [5]. One common ROS that damages cells' DNA and induces lipid peroxidation is hydrogen peroxide (H_2O_2) [6]. Natural antioxidants have a variety of biochemical actions, including as altering intracellular redox potential, scavenging free radicals directly or indirectly, and

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inhibiting the production of reactive oxygen species (ROS) [7]. Numerous antioxidant compounds exhibit anticancer or anticarcinogenic characteristics [8,9].

India is blessed with rich medicinal plant diversity, which would be the best source to obtain a variety of drugs and natural products and some are being used by several tribal communities since ages. Eulophia species comprises a perennial terrestrial orchid (herb) with one to three feet height having fleshy tubers in the form of chain, generally grows along the water channel on deep soil slopes, distributed worldwide and mostly tropical Himalaya and Deccan peninsular region in India [10,11]. It is distributed in tropics and subtropics of world and found throughout the warmer part of India [12,13]. However, with respect to taxonomical knowledge, there is still confusion in identification of species of Eulophia guineensis under the name Amarkand, which has rich food and medicinal value [14,15].

The terrestrial orchid Eulophia guineensis is medium to large in size. They have two or three nodes. The pedicel and ovary are thin, reaching a maximum length of 25 mm (1.0 in), while the floral bracts can reach up to 20 mm (0.8 in) in length and are ovate-lanceolate. The fragrant, waxy blossoms bloom throughout the fall and early winter [16].

Thirty plant species of Eulophia (Family: Orchidaceae) and Dioscorea bulbifera (Family: Dioscoraceae) are generally referred to as "Amarkands." The indigenous people of India frequently use the tubers of the Amarkand group of medicinal plants as both food and medicine. Of these, twenty-one are known to have culinary and medicinal uses [17]. Usually found in semi-natural shrubby-bushy vegetation that is not overly dense, Eulophia guineensis is somewhat shaded from the sun. The surrounding vegetation contained a number of invasive alien plants that we observed [18].

Orchids are distributed throughout the world from tropical to high alpine areas [19]. The Orchid family is with the Asteraceae the two largest families [20]. Eulophia guineensis extract reported that the phytoconstituents, Beta sitosterol, Beta sitosterol glucoside, Beta Amyrin, alkaloids, flavonoids, carbohydrates, steroids, triterpenoids, tannins, phenolics are present in high concentration in the Methanolic extract of Eulophia guineensis. As reported by [35] Falls V et al the concentration of Beta sitosterol & Beta sitosterol glucoside was found to be 88% and 91% respectively in EGE.

2. Objectives

The aim of the present study was to investigate anticancer antioxidant and antibacterial activity of Eulophia guineensis in vitro.

3. Materials and Methods

3.1 Chemicals

Dimethyl sulfoxide (DMSO), propidium iodide (PI), [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] (MTT), [HiMedia, India] , 1,1-diphenyl-2-picrylhydrazyl (DPPH) [Sigma, USA], thiobarbituric acid (TBA), 2,2V-di-p-nitrophenyl5,5V-diphenyl-3,3V-(3,3V-dimethoxy-4,4V-diphenylene)-ditetrazolium chloride (NBT), nicotinamide adenine phosphate (NADPH), xanthine, xanthine oxidase, ethylenediaminetetraacetic acid sodium salt (Na-EDTA), pyridine, sodium azide, glutathione, glutathione reductase, ethidium bromide [HiMedia, India]. All other chemicals were of the highest analytical grade and were purchased from common sources.

3.2 Collection of Amarkand Tubers

Eulophia guineensis tubers were collected randomly during the month of September from Pune, India.

3.3 Authentication of plant species

The leaves and tubers of Eulophia guineensis plant commonly known as Amarkand was collected from area of Harishchandrgad, Pune and authenticated at Botanical Survey of India, Western regional centre,7-Koregaon road, Pune-411001 (Specimen no- SG, DRPEN-1)

3.4 Characteristics

After being divided from other components, the tubers were cleaned, dried, and washed. The macroscopic details of the fresh tubers were documented, including their unique characteristics.

3.5 Preparation of extract

The roots of the plant was taken and shade dried. The coarsely powdered plant of Eulophia guineensis was extracted with methanol at room temperature by

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JCHR (2024) 14(3), 1959-1970 | ISSN:2251-6727

maceration process for 48 h. Extract was dried and concentrated.

3.6 Phytochemical evaluation

After drying, the extracts were weighed. Using the standard test procedures, the presence or absence of various phytoconstituents, such as triterpenoid, steroids, alkaloids, vitamins, tannins, glycosides, and flavonoids was determined [21].

3.6.1 UV-Vis Spectroscopy

UV-Vis spectroscopy was performed using UV-Vis spectrophotometer (Model: Shimadzu 19000i).

The sample (100µl) was raised to 3 ml by adding methanol, and then used to scan the sample in the 200–800 nm range.

3.6.2 IR Spectroscopy

IR Spectroscopy was performed using FT/IR-4100 type A spectrophotometer 8000 series.

The Eulophia guineensis methanol extract was combined with KBr salt and crushed into a thin pellet and then scanning was performed in the 4,000 to 400 cm^{-1} range [22].

3.7. Molecular Docking

Molecular Docking of different phytoconstituents was performed with the help of Autodock vina, Pubchem, Biovia software. Binding affinity of Beta sitosterol, Beta sitosterol glucoside, Beta Amyrin on Estrogen growth factor receptor was determined and was compared with standard drug Tamoxifen.

3.8 Pharmacological screening

3.8.1 Anticancer activity:

Using the MTT test method, the anticancer potential of the methanolic extract of Eulophia guineensis was evaluated in human breast cancer cell line MCF-7. The MCF-7 cell line was procured from National Centre for Cell Science (NCCS), Pune, India. The cells were developed in Dulbecco's Modified Eagles Medium (DMEM), which was enhanced with 10% fetal bovine serum, 100 U/ml of a combination of ampicillin and streptomycin, and 100 mg/ml of penicillin. The cells were grown in humidified incubator supplied with 5% CO_2 and maintained at 37-degree C [23].

3.8.2 Preparation of Plant Extract

The Methanolic Extract of Eulophia guineensis of 10mg was weighed and dissolved into 10ml of distilled water. The solution was vortexed for 10 min and filtered using 0.2 um syringe filter.

3.8.3 Cell viability

The cytotoxicity of the methanolic extract of Eulophia guineensis was assessed using the MTT test. In order to allow for cell attachment, MCF-7 cells (1 x 105 cells/ml) in a complete growth medium were planted into a flat bottom 96-well plate and incubated in CO2 for 24 hours. Different concentrations (1000, 250, 125, 62.5, 31.25, 15.63 & 7.81 ug/ml) of Eulophia guineensis methanolic extract were applied in triplate form to the various wells of a 96-well plate. The control well received no treatment. Following a 24-hour incubation period, each well received 10 uL of MTT solution (5 mg/ml), and the plates were then incubated for 4 hours at 370C in a CO2 incubator. In order to dissolve the MTT crystal in a well, 100 uL of DMSO was added after the supernatant was removed without disturbing the crystal. At the dissolve MTT crystal, the absorbance of the produced purple hue was measured. At 550 nm, the produced purple color's absorbance was measured. To determine the IC50 (minimum concentration of Eulophia guineensis methanolic extract that provides 50% survival of MCF-7 cells), the proportion of cell viability vs concentration was plotted using linear regression interpolation technique [24]

3.8.4 Antioxidant activity

3.8.4.1 Preparation of plant extract

A plant extract stock solution containing 1000 ug/ml was made. 10.65 mg of Eulophia guineensis methanolic extract was weighed and then diluted in 10.65 ml of methanol. After 10 minutes of vortexing, the solution was filtered using a 0.2 um syringe filter.

3.8.4.2 Preparation of DPPH solution

A 100mm stock solution of DPPH solution was made. Using an analytical weighing balance, the necessary amount of DPPH was measured and then dissolved into the necessary volume of methanol. The final concentration of DPPH was obtained by diluting one

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JCHR (2024) 14(3), 1959-1970 | ISSN:2251-6727

millilitre of stock solution with ten millilitres of methanol [25].

3.8.4.3 DPPH Assay

With a few minor modifications, the modified approach was used to assess the plant extract of Eulophia guineensis capacity to scavenge DPPH radicals. A 10mm methanolic solution of DPPH was combined with 1.0ml of an aliquot of test extract at several doses (50,100,200,300,400, and 500 ug/ml) in methanol. After 30 min incubation in darkness at 517nm. The plant extracts' blank absorbance at various concentrations was noted and removed from the test's final absorbance[26].

The percentage inhibition was calculated using the following formula.

Percentage inhibition = (Abs Sample) X 100/ Abs Control IC_{50} values were calculated as the average of triplicate analyses.

3.8.5 Antibacterial activity

The antibacterial activity of methanolic extract of dried tuber powder from Eulophia guineensis was compared with the antibacterial activity of ampicillin, as standard antibiotic. The well diffusion approach was employed. Antibacterial activity was determined against Staphylococcus aureus and Escherichia coli using the well diffusion approach [27,28,29].

4. Results

4.1. Phytochemical Screening

The table no 1 displays observations and findings regarding the qualitative chemical constituents of Eulophia guineensis.

Table No 1: Phytochemical Analysis

Phyto-chemical screening	Eulophia guineensis tubers
Alkaloids	+
Flavonoids	+
Carbohydrates	+
Triterpenoids	+
Tannins	+
Phenolics	+
Saponins	-
Coumarins	-
Anthraquinone glycosides	-

Where, (+) = present, (-) = absent

Chemical constituents in Eulophia guineensis responsible for Anticancer activity Beta sitosterol, Tannic acid, Beta sitosterol glucoside, Beta amyrin, Iupeol, Apigenin, Kaempferol, Iuteolin, Quercetin.

4.2. UV visible spectrum analysis

The qualitative UV-VIS spectrum profile of methanolic extract of Eulophia guineensis showed few sharp peaks from wavelength 200 to 800 nm. The peaks were observed at 202-254nm,270-280nm, 215-265 nm representing Phytosterol, phenolic acid and Tannic acid respectively.

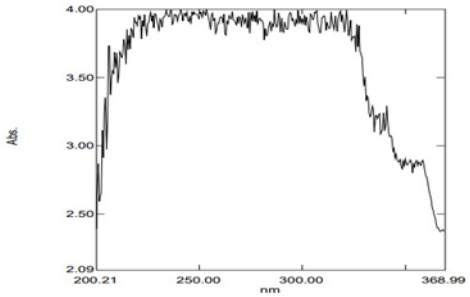

Fig No1: Ultra Violet-Visible Spectroscopy Analysis of Methanolic extract of Eulophia guineensis

4.3. Infrared Spectroscopy analysis

The FT-IR spectrum profile was illustrated in Fig. The FT-IR gave broad peak at 3866.58 cm-1 which indicated the presence of N-H stretching. It gave a strong peak at 3283.7 and 2849.79 cm-1 which indicated the presence of C-H stretching. The peaks obtained at 1727.91, 1611.23 cm -1 indicated the presence of C=O bending. The peak obtained at 1450.21 and 1357.64 cm-1 indicated the presence of C-H Bend out of plane. The peak obtained at 2366.23, 2344.53, 2130.96 cm-1 indicated the presence of C=C stretching. The peaks obtained at 3322.75 cm-1 indicated the presence of Aliphatic primary amines. The FT-IR spectrum confirmed the presence of alcohols, phenols, alkanes, alkynes, alkyl halides, aldehydes, aromatics, nitro compounds and amines in methanolic extract.

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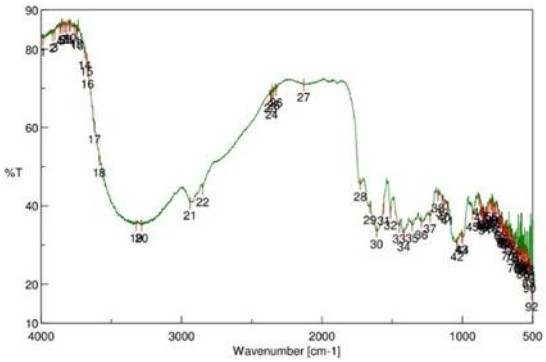

Fig No 2: Fourier-Transform Infrared Spectroscopy analysis of Methanolic extract of Eulophia guineensis

Extract	**Peak value**	**Functional group**	**Functional group name**
Methanolic extract of Eulophia guineensis	3621.66 cm-1 3322.75 cm-1	H-N-C	Aliphatic primary amines
	2366.23 cm-1 2344.53 cm-1 2130.96 cm-1	C=C	Conjugated Alkenes
	1450.21 cm-1 1357.64 cm-1	Polyphenol	Polyphenol skeleton
	1105.98 cm-1 1130.56 cm-1 1727.91 cm-1 1611.23 cm-1	C-O	Carbonyl group
	3283.7 cm-1 2849.79 cm-1	C-H stretching	Alkane
	3866.58 cm-1 3845.85 cm-1	N-H stretching	Amine

Table No 2: Infrared Spectroscopy analysis of Methanolic extract of Eulophia guineensis

5.4. Molecular Docking

5.4.1 Binding affinity of Standard

Mode	Affinity (kcal/mol)	Distance from best mode rmsd l.b	Distance from best mode rmsd u.b
1	-7.3	0	0
2	-6.3	3.707	7.293
3	-6.2	3.986	7.35
4	-6.2	3.712	7.88
5	-6.1	4.379	7.295
6	-6.1	4.125	7.27
7	-6	3.647	7.758
8	-6	3.742	6.539
9	-6	3.846	8.584

Table No 3: Binding affinity of Tamoxifen (Standard)

5.4.2 Binding affinity of Beta Sitosterol

Table No 4: Binding affinity of Beta Sitosterol

Mode	Affinity (kcal/mol)	Distance from best mode rmsd l.b	Distance from best mode rmsd u.b
1	-8.2	0.000	0.000
2	-8.2	2.770	5.314
3	-8.1	2.043	3.447
4	-7.9	3.274	9.578
5	-7.8	2.763	5.387
6	-7.3	3.594	5.789
7	-7.0	5.149	9.594
8	-6.9	5.179	9.356
9	-6.8	1.781	2.480

Journal of Chemical Health Risks

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JCHR (2024) 14(3), 1959-1970 | ISSN:2251-6727

5.4.3 Binding affinity of Beta Sitosterol Glucoside

Table No 5: Binding affinity of Beta Sitosterol Glucoside

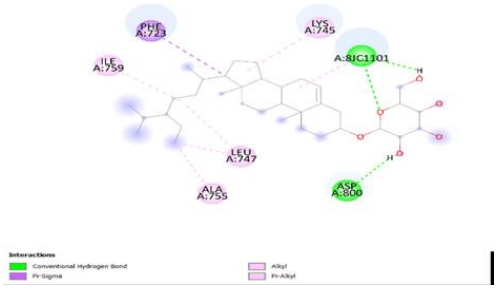
Mode	Affinity (kcal/mol)	Distance from best mode rmsd l.b	Distance from best mode rmsd u.b
1	-9.0	0.000	0.000
2	-8.6	0.688	1.896
3	-8.1	2.338	4.750
4	-8.1	1.168	2.256
5	-8.0	2.207	4.531
6	-7.9	8.247	13.878
7	-7.7	5.820	12.829
8	-7.7	2.152	4.821
9	-7.5	9.132	14.147

5.4.4 Binding affinity of Beta Amyrin

Mode	Affinity (kcal/mol)	Distance from best mode rmsd l.b	Distance from best mode rmsd u.b
1	-8.3	0	0
2	-8	2.629	3.744
3	-7.9	4.256	8.845
4	-7.9	4.459	8.146
5	-7.8	4.498	6.896
6	-7.8	4.007	6.865
7	-7.7	3.225	6.349
8	-7.7	4.256	7.912
9	-7.7	3.957	5.312

Table No 6: Binding affinity of Beta Amyrin

5.4.5 2D Structure of standard and beta sitosterol



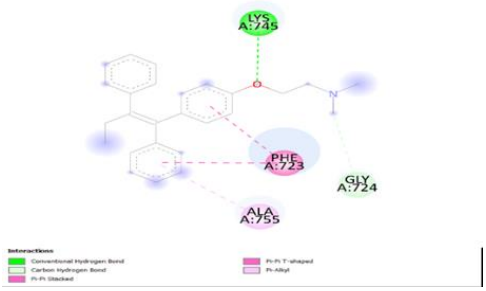


Fig No 3: 2D Structure of standard and beta sitosterol glucoside

Binding affinity of Beta sitosterol glucoside was found more than standard drug Tamoxifen.

5.5 Anticancer Activity

The cell viability checked in triplicate manner and average value was considered

5.5.1 Cell Viability of Column- 1

Table No 7: The Effect of methanolic extract of Eulophia guineensis plant material on MCF-7 cell viability (C1)

Conc. (µg/ml)	Cell Viability (%)
1000	59.89
500	78.81
250	93.78
125	96.58
62.5	96.06
31.25	97.81
15.63	100.53
7.81	101.66

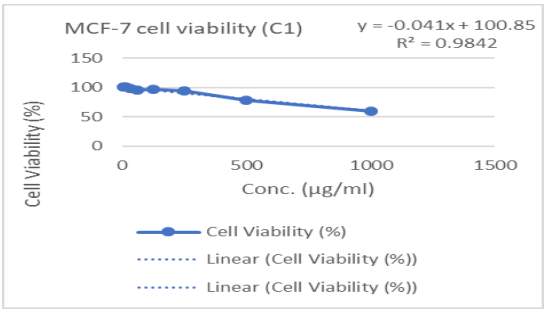


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Figure No 4: The Effect of methanolic extract of Eulophia guineensis plant material on MCF-7 cell viability (C1)

5.5.2 Cell Viability of Column- 2

Conc. (µg/ml)	Cell Viability (%)
1000	68.46
500	84.96
250	88.01
125	89.7
62.5	93.89
31.25	92.44
15.63	93.32
7.81	94.21

Table No 8: The Effect of methanolic extract of Eulophia guineensis plant material on MCF-7 cell viability (C2)

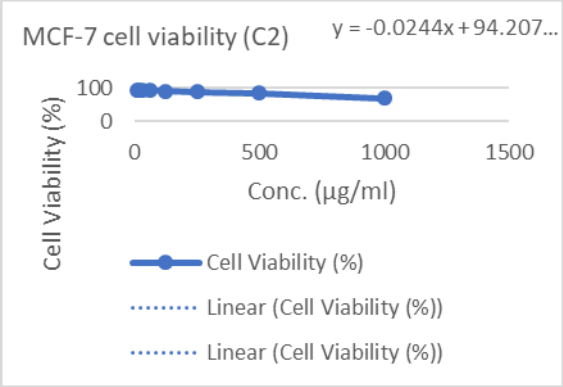

Figure No 5: The Effect of methanolic extract of Eulophia guineensis plant material on MCF-7 cell viability (C2)

5.5.3. Cell Viability of Column- 3

Conc. (µg/ml)	Cell Viability (%)
1000	58.01
500	74.37
250	91.4
125	93.74
62.5	94.16
31.25	96.24
15.63	97.33
7.81	100.33

Table No 9: The Effect of methanolic extract of Eulophia guineensis plant material on MCF-7 cell viability (C3)

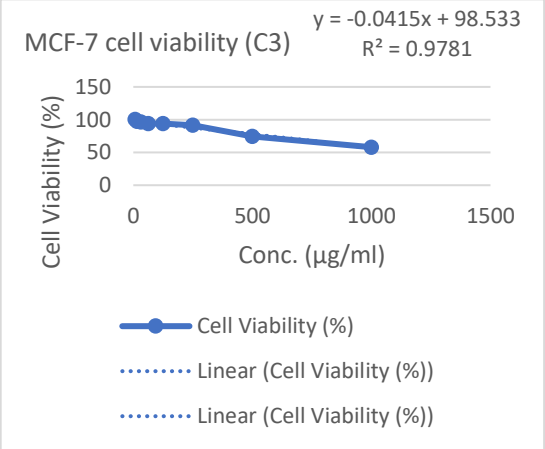

Figure No 6: The Effect of methanolic extract of Eulophia guineensis plant material on MCF-7 cell viability (C3)

Table No 10: The Effect of methanolic extract of Eulophia guineensis plant material on MCF-7 cell viability: Mean IC50 Value

IC50 of C1	1240.24µg/mL
IC50 of C2	1811.72 µg/mL
IC50 of C3	1169.47 µg/mL
Mean	1407.15 µg/mL
SD	352.16

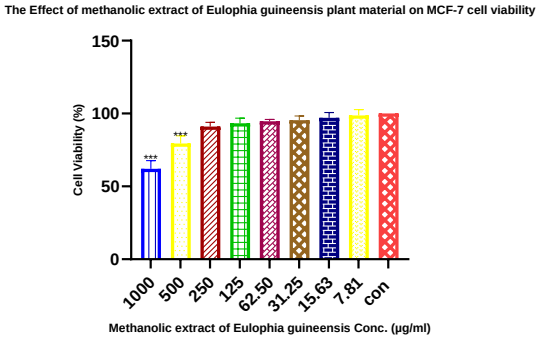

Figure No 7: The Effect of methanolic extract of Eulophia guineensis plant material on MCF-7 cell viability

Journal of Chemical Health Risks
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JCHR (2024) 14(3), 1959-1970 | ISSN:2251-6727

In the present investigation, methanolic extract of Eulophia guineensis was evaluated for anticancer activity using MCF-7 human breast cell line. The test compound was tested at different serially diluted concentrations: 1000, 250, 125, 62.5, 31.25, 15.63 & 7.81 µg/ml in triplicate form. The inhibitory concentrations at 50 (IC50) was found to be 1407.15±352.16 µg/mL. The Eulophia guineensis extract at 1000 ($p<0.001$), 250 ($P<0.01$),125($P<0.05$) µg/ml concentration showed significant decrease in cell viability in comparison to control.

5.6. Antioxidant Activity

5.6.1 Antioxidant activity Expt-01

Table No 11: The Effect of methanolic extract of Eulophia guineensis plant material on DPPH assay

Test Conc. (µg/ml)	Inhibition (%)
50	12.21
100	12.93
200	26.10
300	39.27
400	49.24
500	60.42

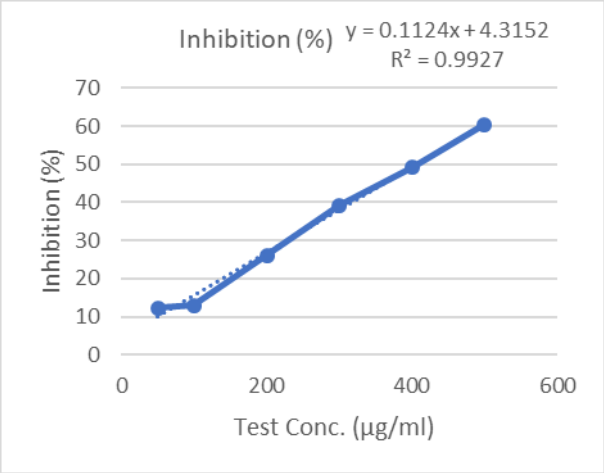

Figure No 8: The Effect of methanolic extract of Eulophia guineensis plant material on DPPH assay

5.6.2. Antioxidant activity Expt-02

Table No 12: The Effect of methanolic extract of Eulophia guineensis plant material on DPPH Assay

Test Conc. (µg/ml)	Inhibition (%)
50	11.41
100	15.56
200	26.90
300	38.55
400	47.65
500	58.66

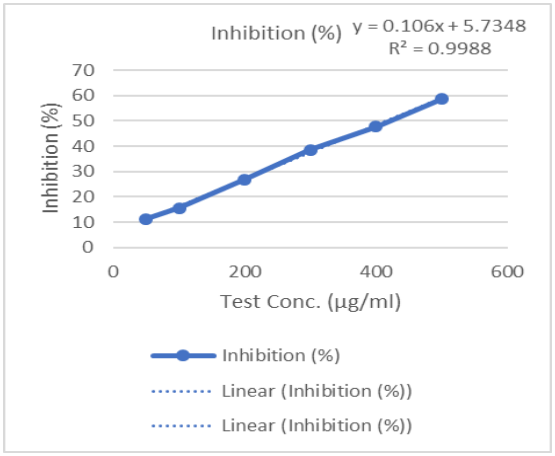

Figure No 9: The Effect of methanolic extract of Eulophia guineensis plant material on DPPH Assay

5.6.3. Antioxidant activity Expt-03

Table No 13: The Effect of methanolic extract of Eulophia guineensis plant material on DPPH Assay

Test Conc. (µg/ml)	Inhibition (%)
50	13.73
100	17.96
200	27.69
300	40.30
400	47.01
500	58.03

Journal of Chemical Health Risks
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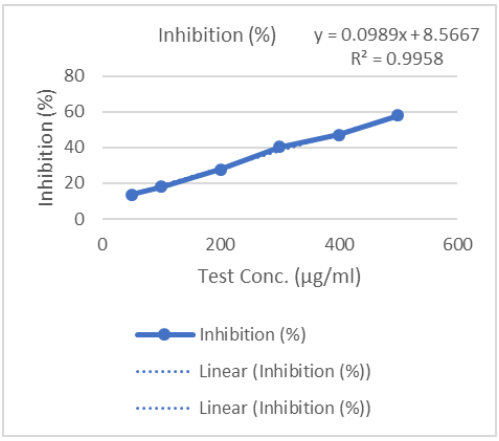

Figure No 10: The Effect of methanolic extract of Eulophia guineensis plant material on DPPH Assay

Table No 14: The Effect of methanolic extract of Eulophia guineensis plant material on DPPH Assay: Mean IC50 Value

IC50 of Expt-01	406.45 µg/mL
IC50 of Expt-02	417.58 µg/mL
IC50 of Expt-03	427.86 µg/mL
Mean	417.30 µg/mL
Standard Deviation	10.71

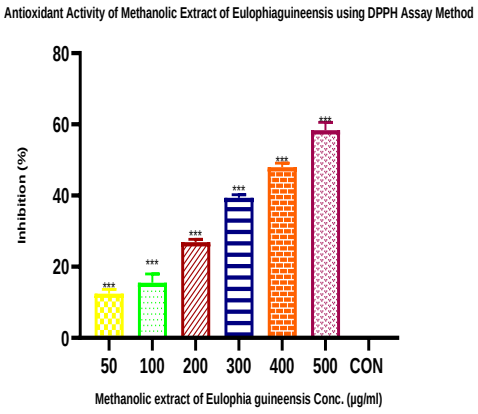

Fig no 11: Antioxidant Activity of Methanolic Extract of Eulophia guineensis using DPPH Assay Method

In the present investigation, methanolic extract of Eulophia guineensis was evaluated for antioxidant activity using DPPH assay method. The test compound was tested at different concentrations: 50, 100, 200, 300, 400 & 500 µg/ml in triplicate manner 2,2 Diphenyl-1-Picrylhydrazyl (DPPH) is a stable organic free radical with an absorption maximum band around 515-528nm and thus, it is a useful reagent for evaluation of antioxidant activity of compounds [30]. In the DPPH test, the antioxidants reduce the DPPH radical to a yellow-coloured compound, diphenyl picrylhydrazine and the extend of the reaction will depend on the hydrogen donating ability of the antioxidants [31]. The effect of methanolic extract of Eulophia guineensis on DPPH radical scavenging activity is shown in Table 11-14 and Figure 8-11. It showed the DPPH radical scavenging activity in a concentration dependent (50-500µg/ml) manner. It showed IC50 value 417.30±10.71µg/ml with the correlation coefficient of 0.9967.

The data suggested the antioxidant activity of Eulophia guineensis against DPPH radicals could be due to presence of Beta Sitosterol glucoside, Beta Sitosterol and tannins. [32,33]

5.7. Antibacterial Activity

Well diffusion method was used against Escherichia coli and Staphylococcus aureus. The methanol extract shows antibacterial activity against S. aureus with maximum zone of inhibition 10 mm and against Escherichia coli zone of inhibition 14 mm.

At 40 µg/L and 60 µg/L concentration maximum zone of inhibition was observed against Staphylococcus aureus. At 40 µg/L concentration maximum zone of inhibition was observed against Escherichia coli.

3. Discussion

Plants are important source of various pharmacologically active molecules. Despite the steadily growing popularity of synthetic products, which has led to the many new drugs that exist today, plant derived compound structures serve as key to drug design and synthesis of new substances for treatment of various disorders. Phytochemicals are considered to be less toxic and more effective [34]. The above research work investigated the potential anticancer activity of a natural extract obtained from the roots of Plant species. Eulophia guineensis extract reported that the phytoconstituents, Beta sitosterol, Beta sitosterol glucoside, Beta Amyrin, alkaloids, flavonoids, carbohydrates, steroids, triterpenoids, tannins, phenolics are present in high concentration in the Methanolic extract of Eulophia guineensis. As reported by [35] Falls V et al the concentration of Beta sitosterol & Beta sitosterol

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1959-1970 | ISSN:2251-6727

glucoside was found to be 88% and 91% respectively in EGE. Thus, we performed characterization and identification tests for phytoconstituents confirmation using IR and UV techniques. According to the UV results a peak was obtained in the range (202-254 nm) confirming presence of phytosterols in the extract, (270-280) confirming presence of phenolic acid in the extract, (215-265) confirming presence of tannic acid in the extract. According to IR results peak obtained within the range (3700-3600 cm-1 OH group, 2790 C-H stretching, 2995cm-1 C=O group) confirmed presence of phytosterols in the extract. Molecular docking study was then performed to confirm anticancer activity of identified phytoconstituent. The results indicated that highest binding score was observed with Beta sitosterol Glucoside.

Our findings reveal compelling evidence supporting the ability of Eulophia guineensis to inhibit the proliferation of cancer cells in vitro (MTT assay), suggesting it as a promising candidate for further development as an anticancer agent [36].

The results of the research study demonstrate an inhibition of cell viability in breast cancer cell line (MCF-7) at doses1000 µg/ml, 500 µg/ml, 250 µg/ml. Elevated rates of reactive oxygen species (ROS) have been detected in almost all cancers, where they promote many aspects of tumor development and progression [37]. Thus, antioxidant study of Eulophia guineensis extract was performed to confirm its antioxidant property. Measurable antioxidant activity was observed at doses 300 µg/ml,400 µg/ml,500 µg/ml.

During chemotherapy, there will be times in your treatment cycle when the number of white blood cells (called neutrophils) is particularly low and you are at increased risk of infection [38]. Thus, we also studied the antibacterial potential of Eulophia guineensis extract and observed that 40 µg/L.

The study reports, anticancer activity (1407.15 µg/mL), antioxidant activity (417.30 µg/mL) of Eulophia guineensis for the first time supported by antibacterial activity. While the exact complete mechanism of action of Eulophia guineensis extract remains to be fully elucidated, the molecular docking studies suggest that it may exert its anticancer effects through EGFR receptor. GFR is found on the surface of some normal cells and is involved in cell growth. It may also be found at high levels on some types of cancer cells, which causes these cells to grow and divide. Anti-EGFR monoclonal antibodies bind to the extracellular domain of EGFR in its inactive state; they compete for receptor binding by occluding the ligand-binding region, and thereby block ligand-induced EGFR tyrosine kinase activation [39][40]. Further investigation into the molecular pathways targeted by Eulophia guineensis extract is required to better understand its mode of action and potential synergy with existing anticancer therapies.

The promising anticancer activity observed in the study requires further evaluation of Eulophia guineensis extract in preclinical animal models and, ultimately, in clinical trials. Due to its natural origin and low toxicity profile in vitro, Eulophia guineensis extract holds promise as a novel therapeutic agent for the treatment of breast cancer. Future studies should focus on confirming its pharmacokinetic properties and evaluating its efficacy through preclinical and clinical studies. While cytotoxic effects of Eulophia guineensis extract were observed in breast cancer cells, we also predict that its specificity for cancer cells versus normal cells will be measurable due the obtained molecular docking results which suggest that Eulophia guineensis methanolic extract act mainly on the Estrogen growth factor receptors (EGF), these EGF receptor present more in proliferative cell as compared to normal cell. Thus, EGE will preferably act on proliferating cells without causing much harm to normal cells [41]. Still further investigation to minimize potential off-target effects will also be required.

Ultimately, our findings path the way for further exploration of Eulophia guineensis extract as a novel therapeutic strategy for breast cancer treatment.

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5. Conflict of interest

The authors declare that they have no conflict of interests.

Journal of Chemical Health Risks

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