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Novel Formazan Derivative: Synthesis, Characterization, Anti-breast Cancer and Antioxidant Investigation

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الخلاصة

تم تخليق مشتق فورمازان جديد **(FOZ)** ودراسته كعامل مضاد لسرطان الثدي وكمضاد للأكسدة. تم إثبات التركيب الكيميائي لـ **FOZ** بواسطة تقنيات **CHN** ، **FT-IR** ، **^{1}H-NMR** و **Mass**. تم تقدير النشاط المضاد للتكاثر لـ **FOZ** تجاه خلايا سرطان الثدي. أظهر المركب المحضر نشاطًا كبيرًا مضادًا لسرطان الثدي في خلايا **(MCF-7)**. أظهر فحص **MTT** أن 16 ميكروغرام / مل من **FOZ** ودوكسوروبيسين كدواء قياسي قمع نمو الخلايا بنسبة 83.33٪ و 92.66٪ على التوالي بعد 48 ساعة. تم تقدير نشاط الفورمازان المضاد للأكسدة مختبريا بتركيزات مختلفة (25-400 ميكروغرام / مل) وأظهرت النتائج أن 400 ميكروغرام / مل من **FOZ** كانت أكثر كفاءة مقارنة بحامض الأسكوربيك.

Abstract

New formazan **(FOZ)** derivative was synthesized and evaluated as an anti-breast cancer and antioxidant agent. Chemical structure of **FOZ** was proved by CHN, FT-IR, ^{1}H-NMR and Mass techniques. The anti-proliferative activity of **FOZ** was estimated toward breast cancer cells. The synthesized compound displayed significant anti-breast cancer activity towered MCF-7 cells. The MTT examination demonstrated that 16 µg/ml of **FOZ** and doxorubicin (**Dox**) as a standard drug suppressed cell growth by 83.33% and 92.66%, respectively after 48hrs. The formazan antioxidant activity was estimated *in vitro* at various concentrations (25-400ug/ml) and the outcomes showed that 400 µg/ml of **FOZ** was more efficient compared to ascorbic acid.

Keywords. Formazan, breast cancer, antioxidant activity, MCF-7

1. Introduction

Cancer is a category of diseases characterized by aberrant cell growth and the capacity to invade or spread to other body regions (1). Breast cancer (BC), which accounts for (12%) of all new instances of cancer each year worldwide, became the most widespread cancer in the world (2). Eight million female alive by the end of 2020 have been diagnosed with BC in the past five years (3). One of the best methods for extending a patient's life is chemotherapy. However, the challenge of drug resistance has put many chemotherapeutic medications in a situation where their therapeutic impact has been decreased (4). Additionally, the harmful effects of chemotherapeutic agents on healthy cells result in unpleasant side effects for the patients. These factors led to increased interest in the

development of new kinds of anticancer medicines that exhibit effective and focused toxicity on tumor cells (5).

Antioxidants inhibit oxidation by reacting with reactive and unstable free radicals to prevent free radical-related diseases like carcinogenesis, cardiovascular and aging (6). A number of endogenous systems pathological states or exposure to a variety of physiochemical conditions, our bodies produce free radicals (reactive oxygen-nitrogen species) (7). There must be equilibrium between antioxidants & free radicals. Oxidative stress is caused by free radicals overpowering the body's capacity to control them. Therefore, free radicals negatively impact DNA, lipids and proteins and cause various disorders in humans. In order to manage this oxidative stress, antioxidants from an external source might be applied (8).

Formazan compounds are an important and colored substances that emanate from the conjugated double bonds and they contain the chain (-N-C=N-NH-) (9). Formazans were extensively researched due to their biological and pharmacological effects such as antibacterial (10), anti-parkinsonian (11), antimicrobial (12), anti-fertility (13), antifungal, anti-hyperglycemic (14), anticonvulsant (15), anti-tubercular (16), anticancer (17) and anti-HIV (18).

In this study, we present synthesis and identification of new formazan compound (**FOZ**). Likewise, cancer cells were utilized in an anti-cancer activity investigation. The research displayed that the **FOZ** has cytotoxic effect against MCF-7. The examination of the antioxidant properties yielded data that supported the **FOZ**'s strong anti-DPPH radical properties.

2. Experimental

2.1. Materials and Instruments

All chemical supplies were bought from Merck and Sigma-Aldrich. FT-IR spectra were collected using a Shimadzu FTIR spectrophotometer. The ^{1}HNMR spectrum was obtained utilizing a Bruker (360 MHz) NMR spectrophotometer. On a FisonsTrio1000 spectrometer, GC-mass spectra were captured. EM-017 analyzer carried out the CHN test.

2.2. Synthesis of *N,N*-Dimethyl-4-((2-phenylhydrazono)methyl)aniline

The Schiffbase was prepared by the reaction of 0.01 mole of 4-(Dimethylamino)benzaldehyde dissolved in 35ml of ethyl alcohol with 0.01 mole of phenylhydrazine with a 2 drops of anhydrous acetic acid. The mixture was refluxed for (4 hrs). Utilizing methanol, the solid product was filtered & recrystallized (19). Pale yellow solid; Yield 73%; m.p. (120-122)^{0}C; R_f = 0.79. **CHN** analysis. **Calc.** C,58.37%; H,3.77%; N,13.96%. **Found**. C,59.01%; H,2.99%; N,13.88. **FT-IR** [cm^{-1}]: *υ*(N-H str.) 3313.82*m*; *υ*(Aromatic C-H str.) 3023.12*m*; *υ*(Aliphatic C-H str.) 2887.53-2802.66; *υ*(C=N) 1599.04*s*; *υ*(N-H bend) 1512.24*s*; *υ*(Aromatic C=C str.)1491.02*s*; *υ*(C-N str.) 1261.49*m*.

2.3. Synthesis of 1,1'-[methylenebis(4,1-phenylene)] bis[3-(4-(dimethylamino)phenyl)-5-phenylformazan]

To prepare the formazan derivative **(FOZ)**, a (1.98 g, 0.01 mol) of cold diazonium chloride of 4,4′-diaminodiphenylmethane was reacted with 0.02 mol of Schiff base dissolved in 20ml of pyridine under stirring for 20 minutes, the mixture temperature was maintained below 5°C. The colored product which was separated is filtered and washed with water & diethyl ether (20). Dark brown solid; Yield 77 %; m.p. (193-195)^{0}C; $\mathbf{R_f}$ = 0.76. **CHN** analysis. **Calc.** C, 72.95; H, 5.22; N, 21.01%. **Found**. C, 73.90; H, 6.06; N, 20.04. **FT-IR** [cm^{-1}]: *υ*(N-H str.) 3026.41*b*; *υ*(Aromatic C-H str.) 3016.25-3002.81*w*; *υ*(Aliphatic C-H str.) 2895.25-2802.66; *υ*(C=N) 1606.76*s*; *υ*(N-H bend) 1518.03*s*,; *υ*(Aromatic C=C str.) 1504.89*s*-1423.52*s* ; *υ*(N=N) 1446.66 -; *υ*(C-N str.) 1357.93*m*$^{-1}$**.** ^{1}**H-NMR:** δ 2.95ppm (*m*, 12H, CH_3-N-CH_3), δ 3.92ppm (*t*, 2H, Ph-CH_2-Ph), δ (6.69-8.57)ppm (*m*, 26H, Ar-H), δ 9.67ppm (*s*, 2H, NH). ***m/z*:** 698 (M^+).

2.4. Antioxidant Activity

The scavenging activity of **FOZ** and vitamin C (standard) were tested utilizing DPPH$^•$ methods. Sample solutions were prepared in different concentrations (25, 50, 75, 100, 200, 300 and 400) µg/ml and added to same volume of DPPH$^•$ (0.1 mM) dissolved in methyl alcohol. The mixture was incubated for 50 min at 25^{0}C. The mixture's absorbance at (517 nm) was tested to determine its antioxidant activity. (21,22). The percentage of inhibition was calculated utilizing following equation:

$$\% \text{ scavenging activity} = (A_{control} - A_{test\ sample} / A_{control}) \times 100$$

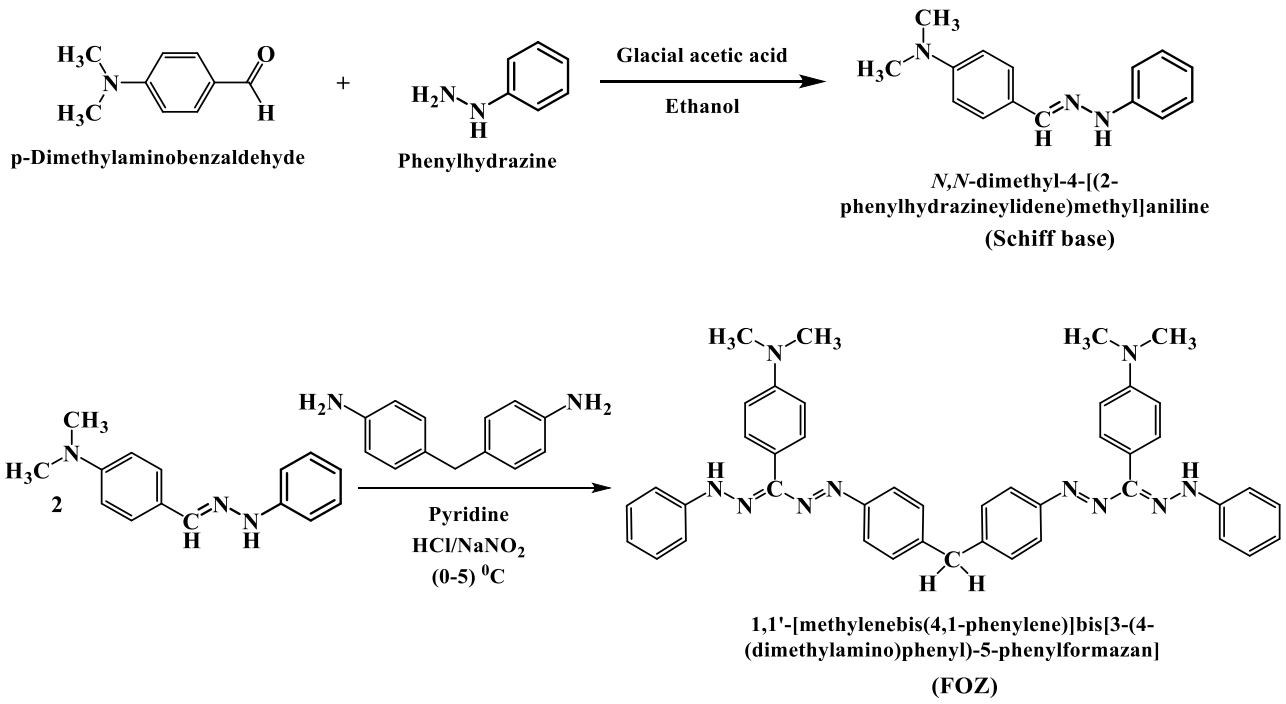
2.5. Anticancer Activity

To measure viability of MCF-7 cells, they were seeded in 96-well plates and treated as needed after 24 hours of seeding. Optical density was assessed at (500 nm), and % cell viability was calculated in comparison to un-treated cells (23,24).

3. Results and Discussion

3.1. Chemistry

Formazan compound **(FOZ)** was synthesized in two phases. According to Scheme 1, the initial step involved producing the Schiff base through reaction of p-Dimethylaminobenzaldehyde with phenyl hydrazine. The imine group was coupled with the diazonium salt of 4,4'-diaminodiphenylmethane in the second step to synthesis the **FOZ**.



Scheme 1: Synthesis of FOZ

3.2. Structure characterization

The structure of **FOZ** was determined basis on the of FT-IR, ^{1}H-NMR, GC-Mass, spectroscopic techniques and CHN analysis.

FT-IR spectrum of *N*,*N*-Dimethyl-4-((2-phenylhydrazono)methyl)aniline explained by the existence of stretching vibrations corresponding to the (HC=N) band at 1599.04 cm^{-1} which is the functional group in the Schiff-base. The *N*,*N*-Dimethyl-4-((2-phenylhydrazono)methyl)aniline also characterized by appearing a medium band due to the stretching vibration of N-H at 3313.82 cm^{-1}. FT-IR of **FOZ** revealed the existence of a new band for stretching corresponding to the (N=N) bonds at 1446.66 cm^{-1}.

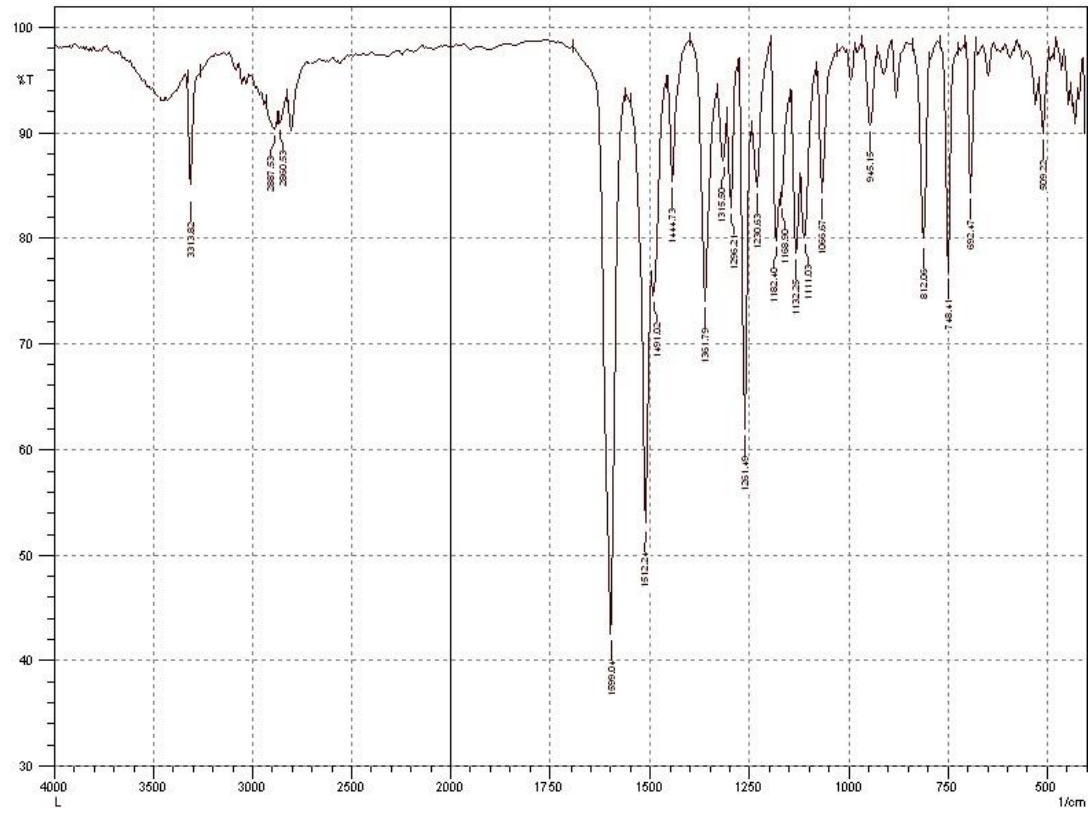


Fig1: FT-IR diagram of *N,N-Dimethyl-4-((2-phenylhydrazono)methyl)aniline*

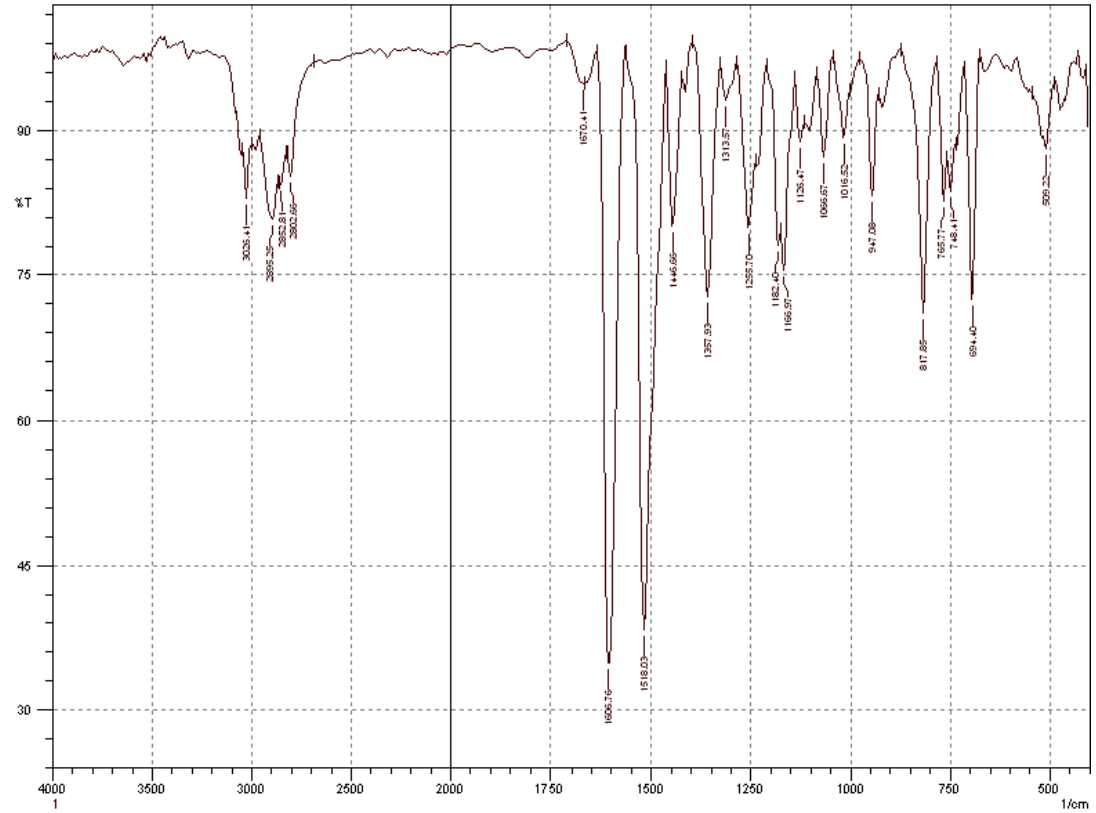


Fig2: FT-IR diagram of FOZ

The **FOZ** formation has been proved by the ^{1}H-NMR. The protons of (N-H) group were recorded in 9.67ppm. The proton of methylene group (Ph-CH_2-Ph) of appeared at δ 3.92ppm. ^{1}H-NMR diagram showed a long and sharp peak at 2.5ppm and 3.3ppm due to DMSO-d_6 solvent.

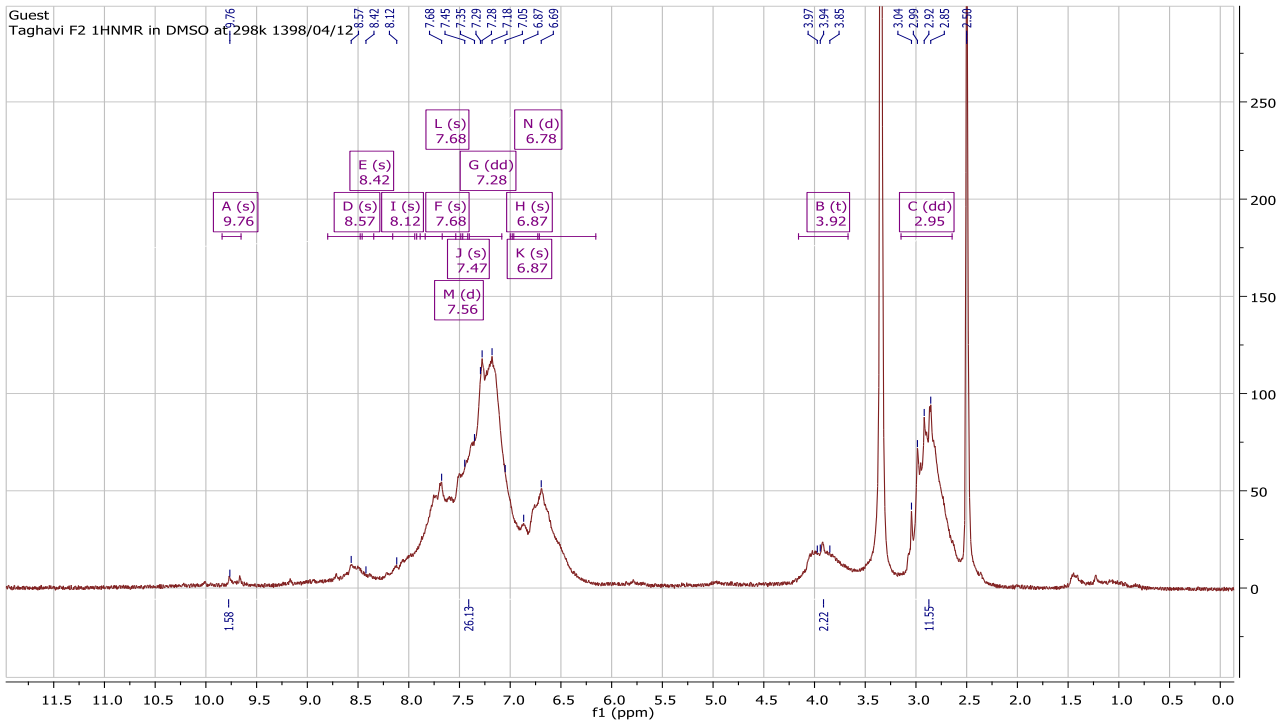


Fig3: ^{1}H-NMR spectrum of FOZ

The structure of formazan was further confirmed by the GC-mass spectra. The GC-mass of **FOZ** showed the correct molecular ion that appeared at m/z: 698 (M+, R%1).

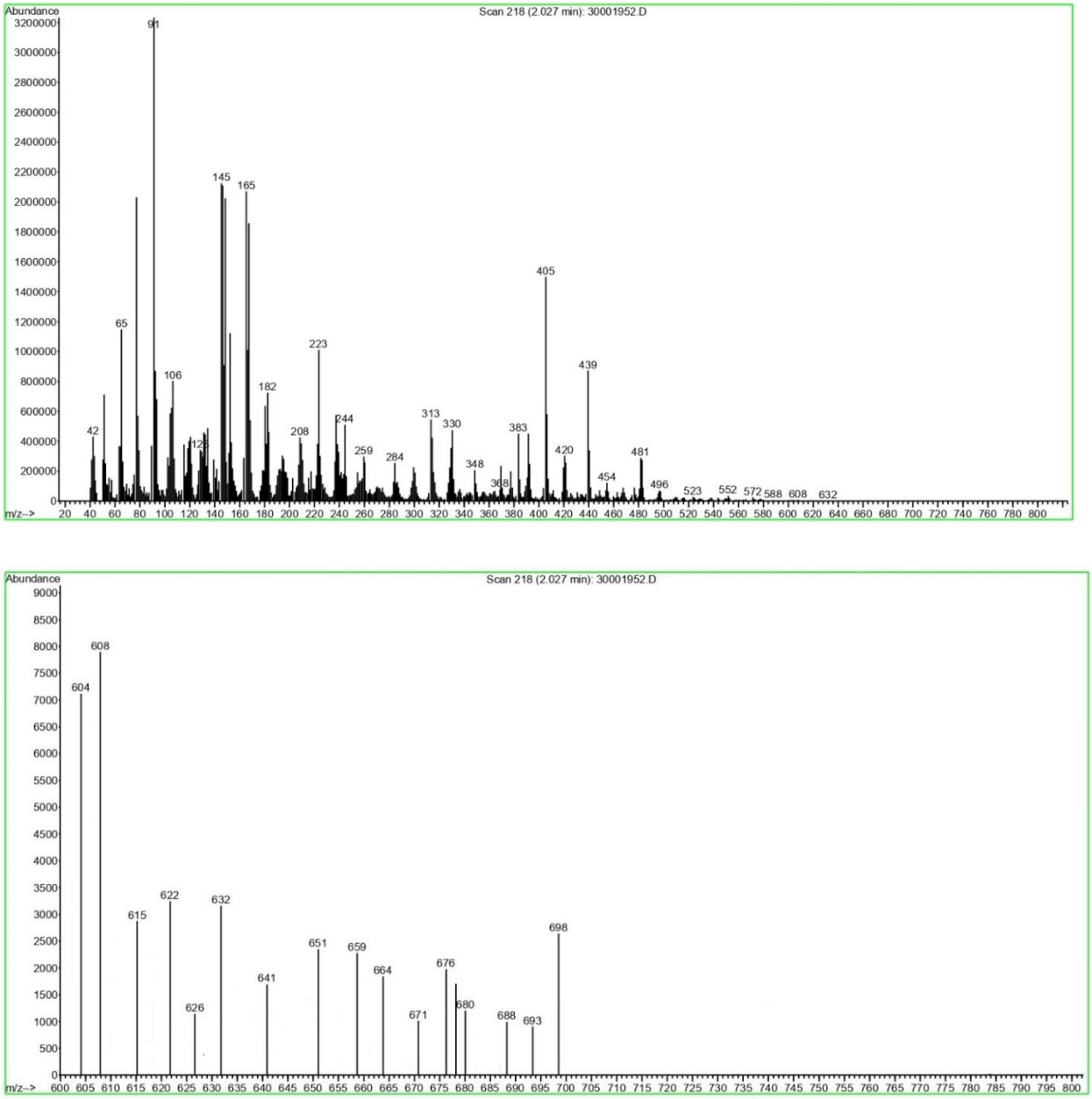


Fig4: Mass spectra of FOZ

3.3. Antioxidant Activity

It is common to quantify the capacity of substances to act as a scavenger for free radicals or to assess antioxidant activity by utilizing a DPPH• radicals that has an absorbance at (517 nm) (25). By donating an electron or H atom, the antioxidant chemicals in the media change the DPPH• into a more stable DPPH molecular product. The antioxidant effect can be assessed spectrophotometrically because the reduced form of DPPH changes from purple to a pale yellow color (26). The results of antioxidant activity of samples were compared to the ascorbic acid (standard) (Fig5). At concentration 25–400 µg/ml, **FOZ** show 20% to

89.33% and ascorbic acid show 26–98% (Table 1). The pi electrons of the aromatic systems in the **FOZ** molecule can compensate for the electron deficiency and prevent oxidation (27).

Table 1. Antioxidant activities results using DPPH• method for FOZ and ascorbic acid.

Conc. (µg/ml)	FOZ	Ascorbic acid
25	20	26
50	29	34.66
75	37.33	45
100	47.33	55.33
200	64	74
300	78	87
400	89.33	98

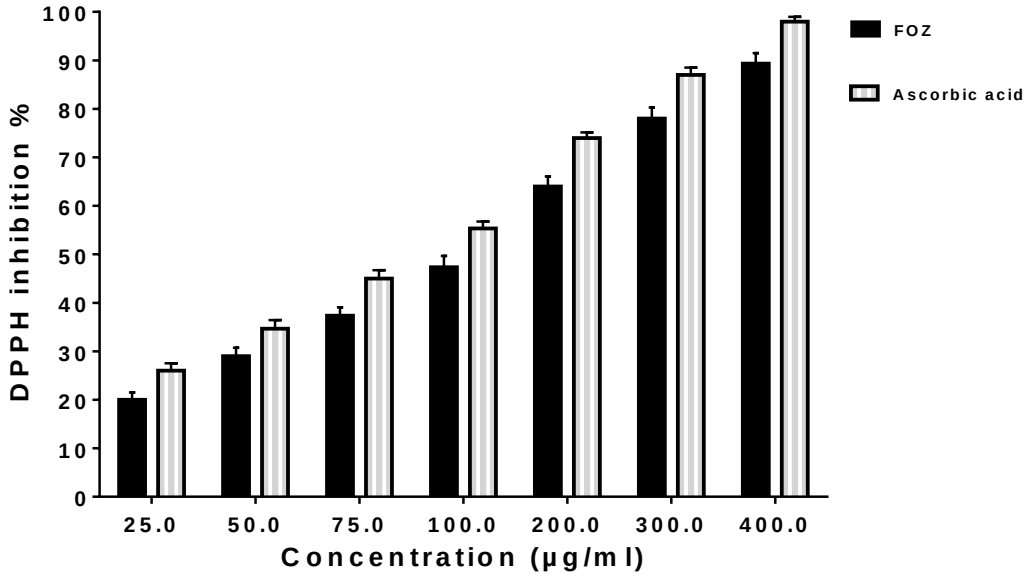


Fig5: The antioxidant activities by DPPH• assay for FOZ and ascorbic acid

3.4. Anticancer Activity

FOZ's antiproliferative efficacy was evaluated against MCF-7 cells at a range of doses (1–16 g/ml). **FOZ** and doxorubicin were found to have IC_{50} values of 3.94 and 4.18 g/ml respectively, after 2 days (Fig.1). The findings revealed that 16 g/ml of doxorubicin and **FOZ** reduced cell growth by 92.66% and 83.33%, respectively. Due to their intercalating or covalent binding capabilities with DNA and interactions with the cell membrane, aromatic compound **FOZ** have shown anticancer effects (28,29).

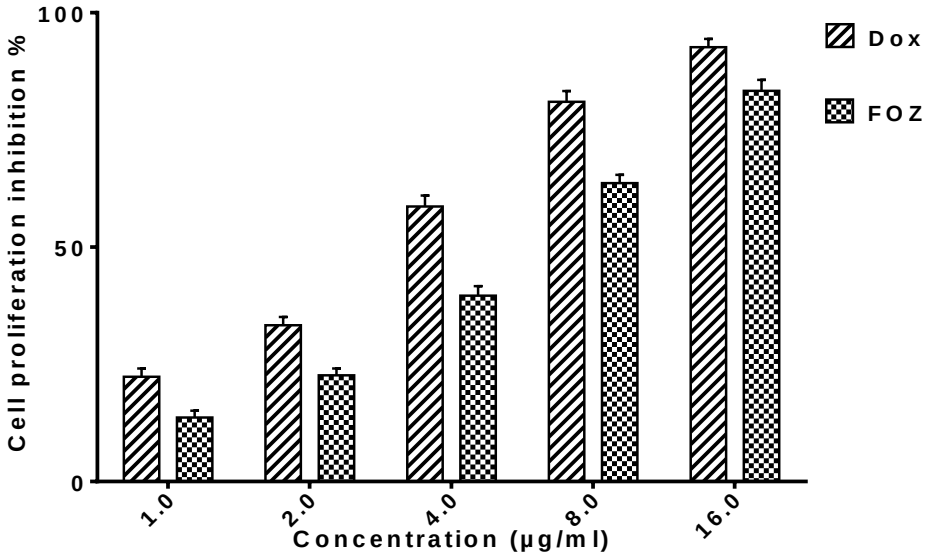


Fig6: Proliferation inhibition % of FOZ and Dox in MCF-7

Conclusions

Successful synthesis of the novel formazan (**FOZ**), which inhibits the growth of breast cancer (MCF-7) cells and has antioxidant properties. Using CHN, FT-IR, 1HNMR, and GC-Mass, the structure of **FOZ** was characterized. Compared to doxorubicin, a common drug, MTT findings for **FOZ** showed good cytotoxicity. Studies on the antioxidant potential have shown that **FOZ** has a strong anti-DPPH radical effect.

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