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Research Article

Active phytoconstituents from *Balanites aegyptiaca* and *Pterocarpus marsupium* and their role in antioxidant defense and cytotoxicity against liver (HepG2) and brain (U87MG) cancer cell lines

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Abstract
Phytochemicals derived from plants offer promising therapeutic potential due to their diverse chemical structures and biological activities. These include alkaloids, terpenes, phenolics and several other natural compounds, which can exhibit synergistic properties in cancer treatment when combined with cancer drugs. Thus, the antioxidant potential and anticancer properties of extracts from two plants, *Balanites aegyptiaca* and *Pterocarpus marsupium* were evaluated *in vitro* in this study against hepatic (HepG2) and brain (U87MG) cell lines to combat cytotoxicity to potential management of disease. The bioactive components responsible for the therapeutic effects were analysed through GC-MS (Gas Chromatography-Mass Spectrometry) analysis. The qualitative and quantitative estimation was done on the methanolic plant extracts, followed by an evaluation of their antioxidant potential using DPPH (2,2-diphenyl-2-picrylhdrazyl) and FRAP (Ferric Reducing Antioxidant Power) assays. The cytotoxicity was determined by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay against cancer cell lines. The extracts of both plants showed potent antioxidant activity with IC_{50} values of 52.78±0.50 µg/ml and 63.19±0.51 µg/ml, respectively, for DPPH assay. The cytotoxicity assay revealed the IC_{50} values as 44.70±0.58 µg/ml against HepG2 cells and, 40.1±0.70 µg/ml against U87MG cells for *B. aegyptiaca* and 59.83±0.47 µg/ml against HepG2 cells and 50.13±3.42 µg/ml against U87MG cells for *P. marsupium*. The GC-MS analysis showed the presence of phytochemicals such as 4-O-Methylmannose, Hexadecanoic acid, 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl, 3,3'-Dimethoxy-4,4'-dihydroxystilbene, cis-Vaccenic acid, 4-(2-Methoxyethyl)phenol, 7-Hydroxyflavanone, etc. The study underscores the significance of examining plant-derived compounds in the context of developing innovative anticancer therapies while highlighting the necessity for in-depth investigations into the phytochemical profiles of these species.

Keywords: Antioxidant, Antiproliferative, *Balanites aegyptiaca*, Cancer, cell lines, *Pterocarpus marsupium*

INTRODUCTION

Cancer is a general term for a range of diseases marked by the unchecked growth and spread of abnormal cells. These rogue cells can invade and damage surrounding tissues and organs, disrupting their normal functions. They can also spread to other areas of the body through the bloodstream or lymphatic system. The pivotal moment in cancer development might not be the transformation of a regular cell into a cancerous one; rather, it is likely the failure of the body's immune cells to detect and eliminate these early cancer cells when they are still scarce in number (Roy and Saikia, 2016). According to the International Agency for Research on Cancer (IARC), more than 20 million cancer cases were reported in the year 2022, which is projected to rise to over 32 million by the year 2050 worldwide (IARC, 2022).

Several factors are likely to significantly impact reducing cancer rates, such as higher intake of fruits and vegetables and better control of infections. Additional factors encompass avoiding excessive sun exposure, increasing physical activity, limiting alcohol consumption, and potentially reducing intake of red meat (Ames and Gold, 1997). Lifestyle modifications such as quitting smoking and adopting a low-fat diet can help lower the likelihood of developing various types of cancers (Sudhakar, 2009).

Cancer is indeed a devastating illness that requires prompt and effective treatment upon diagnosis. In this era, there is a growing demand for novel drugs that are highly efficacious and exhibit lower toxicity and minimal environmental impact. Novel natural products found in plants present promising avenues for innovation in drug discovery (Huang *et al*., 2009).

Several classes of phytochemicals in herbal medicine are increasingly recognized for their therapeutic potential. Cancer patients, in particular, are reported to benefit significantly from these treatments, often experiencing enhanced survivability. Additionally, they are utilized as nutritional supplements due to their anticancer and anti-inflammatory properties, among other therapeutic benefits (Ho *et al*., 2002; Naeem *et al*., 2022; Yang *et al*., 2025).

Phytochemicals are derived from the primary and secondary metabolites present in plants. The distinction between primary and secondary metabolism is not clearly defined. Moreover, these classifications are intertwined because primary metabolism provides the initial substrates that form the basis for secondary metabolic pathways. Secondary metabolites are characterized by unique features such as limited production and diverse structures. The chemical structures of secondary metabolites are notably diverse, with many displaying highly complex compositions (Naji *et al*., 2024).

The plant's secondary metabolites are divided into 3 classes based on their chemical structure. Alkaloids are nitrogen-containing compounds with a heterocyclic ring, terpenes containing isoprene units (branched 5 carbon skeleton), and phenolic compounds having hydroxyl group with an aromatic ring (Anulika *et al*., 2016). Out of these classes, phenolic compounds are the most diverse and present in almost all plant parts.

Balanites aegyptiaca L. Delile, also known as desert date or hingot, belongs to the family Zygophyllaceae. It is a plant native to semi-arid and arid regions, particularly found in desert areas across Africa and parts of Asia. The desert date tree is known for its hardiness and ability to thrive in harsh desert conditions. The fruits of *B. aegyptiaca* are edible and have various traditional uses, including food, medicine, and cosmetics (Al-Thobaiti and Abu Zeid, 2018).

B. aegyptiaca is known for containing several active bioconstituents, such as saponins, flavonoids, triterpenoids, etc., that contribute to its medicinal properties. These bioconstituents collectively give *B. aegyptiaca* a range of medicinal properties, including antioxidant, anti-inflammatory, antimicrobial, and possibly anticancer effects. Traditional uses of the plant include treating skin conditions and gastrointestinal disorders and even as an anthelmintic (to expel parasitic worms) (Saboo *et al*., 2014; Tesfaye, 2015; Zein *et al*., 2024).

Pterocarpus marsupium Roxb., commonly known as Vijayasar, belongs to the Fabaceae family and is a tree native to the Indian subcontinent. It is renowned for several medicinal properties, particularly associated with its heartwood. The plant is recognized for being a valuable source of bioactive chemical components like alkaloids, tannins, flavonoids, saponins, phenolic compounds, fats and fixed oils (Devgun *et al*., 2009; Ahmad and Rajagopal, 2015).

In traditional medicine, particularly in Ayurveda, *P. marsupium* has been utilized for centuries to address a range of ailments due to its diverse pharmacological properties such as anti-inflammatory, astringent, antitumour, antihelmintic, anti-diabetic and many other (Katiyar *et al*., 2016; Umamaheswari *et al*., 2023).

Therefore, the goal of this study was to evaluate the antioxidant and anticancer properties of raw/crude methanolic extracts from two plants, namely *Balanites aegyptiaca* and *Pterocarpus marsupium*, against human hepatoma (HepG2) and glioblastoma (U87MG) cell lines and to identify the potential natural components present in them.

MATERIALS AND METHODS

Plant material collection and authentication

Balanites aegyptiaca fruits were sourced from the vicinity of Jaipur, Rajasthan, India, while *P. marsupium* heartwood was obtained from the local market of Rohtak, Haryana, India. Authentication and certification of these plant materials were conducted by the Raw Materials Herbarium and Museum, Delhi, CSIR-RHMD, NIScPR with NIScPR/RHMD/Consult/ 2022/ 4019-20-2 (*Balanites aegyptiaca*) and NIScPR/RHMD/Consult/ 2022/ 4019-20-4 (*Pterocarpus marsupium*) authentication numbers.

Plant extract preparation

The fruit pulp and heartwood were air-dried in the shade until completely dehydrated, after which they were finely powdered using an electric grinder. Next, 250 grams of the powdered plant material was mixed into 2.5 liters of methanol and placed on a shaker for 72 hours. After the extraction period, the resulting mixture was filtered using Whatman Filter Paper No. 1 to obtain a clear filtrate. The filtrate was then concentrated into a slurry using a Rotatory Vacuum Evaporator. This slurry underwent further drying in an oven set at 60°C until a dried crude methanolic extract was obtained. Finally, these extracts were kept at 4°C for future experimental use.

Qualitative tests

The qualitative analysis was made to identify specific active chemical components such as alkaloids, terpenoids, phenols, flavonoids, reducing sugars, tannins, and saponins using the methodologies, with some adjustments (Sasidharan *et al*., 2011).

Quantitative tests

Total phenolic content

The TPC of extracts were assessed using the FC (Folin –Ciocalteu) reagent method (Brahmi *et al*., 2017), with slight changes. Specifically, 1 ml plant extract was combined with 2 ml FC reagent (diluted with distilled water 1:10 v/v) and 1 ml of 20% sodium bicarbonate (Na_2CO_3). The resulting mixture was incubated at 40°C for 30 minutes, after which the OD (Optical density) was measured at 765 nm. A standard curve was generated using different concentrations of gallic acid. The sample's total phenolic content (TPC) was expressed in mg of gallic acid equivalents (GAE) per gram. Triplicate measurements were conducted to ensure precision.

Total flavonoid content

With minor adjustments, the Aluminium Chloride technique (Anokwuru *et al*., 2011) was used to calculate the TFC (total flavonoid content) in the methanolic extracts. First, 0.3 millilitre of 5% sodium nitrite ($NaNO_2$), 4 millilitres of distilled water, and 1 millilitre of plant extract were combined. 0.3 ml of 10% aluminium chloride ($AlCl_3$) was added, and the mix was incubated for an additional 5 minutes after the initial 5 minutes of incubation. After adding 2 ml 1M sodium hydroxide (NaOH), the mixture was allowed to sit at room temperature for 30 minutes. The absorbance at 510 nm was then measured. Using different quantities of quercetin (1000, 500, 250, 125, 62.5, and 31.25 µg/ml), a standard curve was created. The sample's total flavonoid content was measured in milligrammes of quercetin equivalents (QE) per gram. Triplicate experiments were conducted for accuracy.

Antioxidant tests

2,2-diphenyl-2-picrylhdrazyl (DPPH) assay

The antioxidant activity of the plant extracts was assessed using the DPPH method (Uddin *et al*., 2022), with minor modifications. Specifically, 0.4 ml of plant extract at various concentrations was combined with 3.6 ml of 0.1 mM DPPH solution. The solution was then incubated in the dark for 30 minutes at room temperature before measuring the absorbance at 517 nm. Methanol was the control, while ascorbic acid was the standard antioxidant. Each test was performed in triplicate. The Radical Scavenging Activity (RSA) was analysed using the given formula :

RSA (Radical Scavenging Activity)= $Absorbance_{(Control)} - Absorbance_{(Sample)} / Absorbance_{(Control)}$ Eq.1

Ferric Reducing Antioxidant Power (FRAP) assay

With few changes, the FRAP test (Dutta and Ray, 2020) was used to assess the plant extracts' antioxidant capability. Different plant extract concentrations were combined with 1% potassium ferricyanide ($K_3Fe(CN)_6$) and 0.2 M phosphate-buffered saline (PBS) (pH 6.6). Following a 20-minute incubation period at 50°C, 10% Trichloro acetic acid (TCA) ($C_2HCl_3O_2$) was mixed and centrifuged for 10 minutes at 3000 rpm. The OD was then measured at 700 nm using 1.5 ml supernatant, 1.5 ml distilled water, and 0.1 ml 0.1% ferric chloride ($FeCl_3$). Ascorbic acid was used as the standard, while methanol was used as the control instead of the plant extract. Each test was run three times to ensure accuracy and reliability.

Cell culture

The hepatocellular carcinoma (HepG2) and glioblastoma (U87MG) cell lines were procured from the National Centre for Cell Science (NCCS), Pune, India. The cells were provided with minimum essential medium (MEM) with 10% fetal bovine serum (FBS) and 1% Penicillin/ Streptomycin antibiotic solution (v/v). Growth conditions included a 5% CO_2 atmosphere, 95% humidity and a temperature of 37°C.

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) Assay

The antiproliferative activity of HepG2 and U87MG cells was evaluated using the MTT assay with some modifications (Mosmann, 1983). During their exponential growth phase, cells were seeded at a density of $5x10^4$ cells in 100 µl medium per well in a 96-well plate and allowed to adhere for 24 hours under standard growth conditions (5% CO_2, 95% humidified air, 37°C). After 24 hours, varying concentrations of crude plant extracts in the medium were added, and the cells were incubated again for 24 hours. MEM was the negative control, and paclitaxel (1 µM) served as the positive control. After incubation, the medium was discarded, 10 µl of MTT dye was added to each well, followed by a 4-hour incubation in the dark. Then, 100 µl of solubilizing solution was added to dissolve the formazan crystals. OD was read at 570 nm using a UV-Vis ELISA plate reader within 1 hour. The experiment was performed in triplicate, and the optical densities were used to calculate the percentage viability of the cells as follows :

$$\text{Cell Viability} = \frac{Absorbance_{(Sample)} - Absorbance_{(Blank)}}{Absorbance_{(Control)} - Absorbance_{(Blank)}} \quad \text{Eq. 2}$$

GC-MS (Gas Chromatography-Mass Spectrometry) analysis

The GC-MS analysis of the crude methanolic extract of *B. aegyptiaca* and *P. marsupium* was conducted using a Shimadzu GC-MS QP 2020 gas chromatograph. The analysis utilized a RXI5 SILMS column from Restek, USA, with dimensions of (30mx0.25µmx0.25mm) (lengthfilm x thickness x internal diameter). To prepare the sample, 1 milligram of the extract was dissolved in 1 milliliter of methanol, and a 1 microliter aliquot was

injected into the GC-MS instrument in split mode. Helium was the carrier gas at a flow rate of 1 mL/minute.

During analysis, the temperature of the column was initially held at 50°C for 1 minute. Subsequently, the temperature ramped to 250°C at a rate of 8°C per minute and maintained at 280°C for 3 minutes. The temperatures for the injector and detector were adjusted to 230°C and 280°C, respectively.

In the MS operation, the ionization energy was 70 eV, and the ion source temperature was 230°C. The identification of components was based on matching their fragmentation spectra with entries in the NIST 2017 library.

Statistical analysis

Results were calculated as Mean±SEM. Statistical analysis was conducted employing Student's t-test and One-way ANOVA, followed by Dunnette's test, utilizing Graphpad Prism software 8.0.

RESULTS

Qualitative and quantitative tests

The methanolic extract of *B. aegyptiaca* and *P. marsupium* underwent phytochemical screening, indicating the presence of alkaloids, terpenoids, phenols, flavonoids, reducing sugars and tannins (Table 1). Notably, the heartwood extract of *P. marsupium* lacked saponins, while the fruit extract of *B. aegyptiaca* showed the presence of saponins.

The TPC in *B. aegyptiaca* fruit was found to be 46.89±0.59 mg of GAE/g and that of *P. marsupium* heartwood was 37.11±0.68 mg of GAE/g. The TPC in *B. aegyptiaca* was found to be significantly slightly higher than that of TPC in *P. marsupium*. The TFC in *B. aegyptiaca* fruit was found to be 28.67±0.36 mg of QE/g and that of *P. marsupium* heartwood was 20.17±0.36 mg of QE/g. The TFC in *B. aegyptiaca* was significantly higher than that of TFC in *P. marsupium* (Table 2).

DPPH (2,2-diphenyl-2-picrylhdrazyl) assay

The antioxidant capacity of both plants was examined by measuring the %RSA based on the absorbance of plant extracts and ascorbic acid across different concentrations. It was observed that the *B. aegyptiaca* extract exhibited significantly greater RSA compared to *P. marsupium* extract across all concentrations. Ascorbic acid showed maximum RSA compared to both the plants (Table 3 and Fig. 1). The half maximal inhibitory concentrations i.e., IC_{50} value for the plant extracts, were determined by the regression curve analysis and it was found that the IC_{50} value of Ascorbic Acid 32.61±4.10 µg/ml was lowest followed by *B. aegyptiaca* 52.78±0.50 µg/ml and *P. marsupium* 63.19±0.51 µg/ml (Table 4).

FRAP (Ferric Reducing Antioxidant Power) activity

In the FRAP assay, the antioxidants in the sample reduce a Fe^{3+} (ferric) ion to a Fe^{2+} (ferrous) ion. The assay can indeed be used to measure the ascorbic acid content in a sample. Ascorbic acid, also referred to as Vitamin C, is a powerful antioxidant that can reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) in the FRAP assay. A calibration curve using known ascorbic acid concentrations was prepared using the FRAP assay to determine the ascorbic acid content. The reducing power of *B. aegyptiaca* and *P. marsupium* extracts, evaluated by determining the content of ascorbic acid in both the extracts, was found to be 47.42±0.36 and 32.42±0.60 µmol AAE/g respectively (Table 5).

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay

The MTT assay employed to assess cell viability following treatment with varying concentrations of methanolic extracts from *B. aegyptiaca* and *P. marsupium* revealed concentration-dependent cytotoxic effects of both *B. aegyptiaca* and *P. marsupium* methanolic extracts on HepG2 (Table 6 and Fig. 2) and U87MG cells (Table 7 and Fig. 3). The viability of both cancer cell lines decreased significantly with increasing concentrations of the extracts. However, the potency of the extracts varied between the two cell lines and between the two plant species, suggesting differential sensitivity to the bioactive compounds found in the extracts.The IC_{50} values of both the plant extracts against HepG2 and U87MG cells were found to be 44.70±0.58µg/ml; 40.1±0.70 (*B. aegyptiaca*) and 59.83±0.47 µg/ml; 50.13± 3.42 µg/ml (*P. marsupium*) respectively.

Table 1. Phytochemicals present in *Balanites aegyptiaca* and *Pterocarpus marsupium* methanolic extracts

S. No.	Test for	*Balanites aegyptiaca*	*Pterocarpus marsupium*
1.	Alkaloids	+	+
2.	Terpenoids	+	+
3.	Phenols	+	+
4.	Flavonoids	+	+
5.	Reducing Sugars	+	+
6.	Tannins	+	+
7.	Saponins	+	-

Table 2. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) in *Balanites aegyptiaca* and *Pterocarpus marsupium*

S. No.	Extract	TPC (mg GAE/g)	TFC (mg QE/g)
1.	*Balanites aegyptiaca*	46.89±0.59**	28.67±0.36**
2.	*Pterocarpus marsupium*	37.11±0.68**	20.17±0.36**

Results are presented as Mean±SEM being significant statistically via student's t-test **(p<0.01)

Table 3. Radical Scavenging Activity (RSA) of Ascorbic acid, *Balanites aegyptiaca* and *Pterocarpus marsupium* at different concentrations

S. No.	Sample	Concentration (µg/ml)				
		62.5	125	250	500	1000
1.	Ascorbic Acid	45.56±0.44	54.98±0.43	66.11±0.24	79.40±0.22	98.98±0.05
2.	*Balanites aegyptiaca*	45.12±0.06^{ns}	52.30±0.15^{ns}	63.94±0.03^{ns}	77.47±0.02^{ns}	92.50±0.24^{ns}
3.	*Pterocarpus marsupium*	44.41±0.04**	52.17±0.08**	63.11±0.01**	75.25±0.09**	90.16±0.00**

Results are presented as Mean±SEM being significant statistically via One-way ANOVA **(p<0.01) and ^{ns}(Not significant)

Table 4. IC_{50} concentrations of Ascorbic acid, *Balanites aegyptiaca* and *Pterocarpus marsupium*

S. No.	Sample	IC_{50} (µg/ml)
1.	Ascorbic Acid	32.61±4.10
2.	*Balanites aegyptiaca*	52.78±0.50**
3.	*Pterocarpus marsupium*	63.19±0.51***

Results are presented as Mean±SEM being significant statistically via One-way ANOVA.**(p<0.01) and *** (p<0.001)

Table 5. Ascorbic acid content in *Balanites aegyptiaca* and *Pterocarpus marsupium* determined by FRAP assay

S. No.	Sample	FRAP (µmol AAE/g)
1.	*Balanites aegyptiaca*	47.42±0.36***
2.	*Pterocarpus marsupium*	32.42±0.60***

Results are presented as Mean±SEM being significant statistically via student's t-test *** (p<0.001)

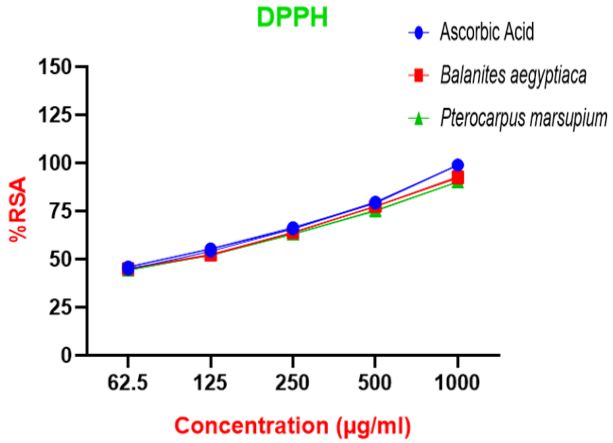

Fig. 1. *Graph showing Radical Scavenging Activity (RSA) of Ascorbic acid, Balanites aegyptiaca and Pterocarpus marsupium at different concentrations*

Table 6. Cell viability determined by MTT assay against HepG2 cells at different concentrations of plant extract

S. No.	Sample	Concentration (µg/ml)							
		Control	3.125	6.25	12.5	25	50	100	Paclitaxel 1 µM
1.	*Balanites aegyptiaca*	100±0	94.56±0.47*	84.26±0.95*	73.47±0.58*	58.12±0.62*	49.97±0.35*	35.47±1.08*	33.65±1.18**
2.	*Pterocarpus marsupium*	100±0	96.52±0.19*	85.19±0.19*	73.71±0.41*	61.47±0.23*	57.35±0.13*	40.57±0.11*	33.65±1.18**

Results are presented as Mean±SEM being significant statistically via One-way ANOVA *(p<0.05) and **(p<0.01)

Table 7. Cell viability determined by MTT assay against U87MG cells at different concentrations of plant extract

S. No.	Sample	Concentration (µg/ml)							
		Control	3.125	6.25	12.5	25	50	100	Paclitaxel 1µM
1.	*Balanites aegyptiaca*	100±0	87.29±0.56**	76.78±0.52**	65.54±0.20**	56.41±1.68**	46.52±0.46**	38.51±1.42**	35.91±0.26**
2.	*Pterocarpus marsupium*	100±0	92.76±0.60**	79.46±1.22**	67.68±0.22**	59.79±0.31**	47.54±0.40**	43.01±1.66**	35.91±0.26**

Results are presented as Mean±SEM being significant statistically via One-way ANOVA **(p<0.01)

GC-MS analysis of plant extracts

The GC chromatogram (Fig. 4) revealed the presence of 64 compounds in *B. aegyptiaca* fruit extract out of which some major compounds were found to be 4-O-Methylmannose, 3,4,5,6,7,9-Hexahydro-2H-xanthene-1,8-dione, Propanoic acids, 5-Hydroxymethyl-2[5H]-furanone, Furanmethanol, 1,2-Butanediol, 1,2-Oxazine,tetrahydro-2-methyl, Furanone, 2,5-Furandione, 3-methyl, 9,12-Octadecadienoic acid, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6, O-Methylisourea, 2-octanol, 5-Hydroxymethylfurfural, Butanedioic acid, 9, 12, 15-Octadecatrienoic acid, 2-hydroxy-2-methyl, 1,2,3-Propanetriol,1-acetate, 1,3-Propanediol, 2-(hydroxymethyl)-2-nitro, Hexadecanoic acid, etc. The major peaks found in the chromatogram are mentioned in Table 8, along with their retention time and molecular formula.

The chromatogram (Fig. 5) revealed the presence of 41 compounds in *Pterocarpus marsupium* heartwood extract which includes compounds such as Hexanoic acid, 3,5-Dimethoxybenzyl alcohol, 2-Naphthalenemethanol, Benzoic acid, 4-hydroxy-3,5-dimethoxy,Tricyclo (2,7) dec-8-ene-3-methanol, 3,7-Cyclodecadiene-1-methanol, n-Hexadecanoic acid, 9,12-Octadecadienoic acid, cis-Vaccenic acid, 4-(2-Methoxyethyl)phenol, 7-Hydroxyflavanone, Phenol, 4-(2-aminoethyl), 3,3'-Dimethoxy-4,4'-dihydroxystilbene, Propanoic acid, Benzofuran, 2,3-dihydro, 1-Pentadecene, etc. The major peaks found in the chromatogram are listed along with their retention time and molecular formula (Table 9).

DISCUSSION

Herbal medicines, derived from plant materials or preparations, represent the oldest known form of health care. It encompasses the use and study of medicinal herbs to avoid and manage illnesses and enhance well-being. In line with the World Health Organization (WHO), herbal medicines are completed products with labels that include active ingredients sourced from plant parts or other plant materials. Several anticancer drugs, such as Paclitaxel, Vinblastin, Vincristine, and Camptothecin, are originally derived from plant sources. Such drugs are extracted from plant sources through secondary metabolites like phenols, alkaloids, fatty acids, terpenes, and more (Atmakuri and Dathi, 2010; Moraes *et al*., 2017).

The results of the present study demonstrated the presence of several such phytoconstituents in both plant extracts. The methanolic extract of *B. aegyptiaca* and *P. marsupium* indicated the presence of alkaloids, phenols, flavonoids, reducing sugars, tannins and terpenoids. The presence of saponins was also indicated in *B. aegyptiaca* fruit extract. The Total Phenolic Content (TPC) for *B. aegyptiaca* fruit extract in this study was found to be 46.89±0.59 µg/ml, while a TPC of 245±8.1 µg/ml was reported for the *B. aegyptiaca* plant collected from the Aleg site of Mauritania in Africa in another study. The Total Flavonoid Content (TFC) of *B. aegyptiaca* in present research was 28.67±0.36 µg/ml, compared to 28.8±5.4 µg/ml reported for the same (Abdelaziz *et al*., 2020). The antioxidative activity, analysed by the DPPH assay, yielded IC_{50} values of 32.61±4.10 µg/ml for Ascorbic Acid, 52.78±0.50 µg/ml for *B. aegyptiaca*, and 63.19±0.51 µg/ml for *P. marsupium*. In a study, the IC_{50} value obtained for *B. aegyptiaca* leaf methanolic extract by DPPH assay was found to be 40.08 µg/ml (Hassan *et al*., 2016). Similarly, the IC_{50} value evaluated for ascorbic acid and *P. marsupium* heartwood methanolic extract was 34.0 and 53.0 µg/ml, respectively (Tippani *et al*., 2010).

The cytotoxicity assay revealed the IC_{50} values as 44.70±0.58 µg/ml against HepG2 cells and, 40.1±0.70 µg/ml against U87MG cells for *B. aegyptiaca* and 59.83±0.47 µg/ml against HepG2 cells and 50.13±3.42 µg/ml against U87MG cells for *P. marsupium in this study*. In several other studies, the half maximal inhibitory concentration of *B. aegyptiaca* ethanolic fruit extract was determined as 60 µg/ml against breast cancer cells (MCF-7), and *B. aegyptiaca* methanolic cell suspension showed IC_{50} values of 82.7 and 41.2 µg/ml against HepG2 and PC-3 cancer cells (Al-Malki *et al*., 2016; Sherif and Emara, 2016). In another study, the proliferation of Hela and PC-3 cells was inhibited by the ethanolic extract of *P. marsupium* at concentrations 59.87 and 79.02 mg/ml (Vijayarekha *et al*., 2023). The effect of a phytocomponent pterostilbene found in *P. marsupium* was determined to induce cytotoxicity in HepG2 cells by MTT assay. It was found that the component reduced the viability of HepG2 cells in a dose-dependent manner, revealing the IC_{50} value of 74 µM (Khalil *et al*., 2023). Further, the studies on glioblastoma cells are very limited. The present study is the first to show the effects of the methanolic extracts of *B. aegyptiaca* and *P. marsupium* on the U87MG cell line.

The aforementioned abilities of the methanolic extracts of *B. aegyptiaca* and *P. marsupium* to combat oxidative stress and inhibit cell growth may be attributed to the active phytoconstituents identified by the GC-MS analysis. Components with major peaks such as 4-O-Methylmannose, 3,4,5,6,7,9-Hexahydro-2H-xanthene-1,8-dione, n- Hexadecanoic acid, 2-Naphthalenemethanol, 9, 12, 15-Octadecatrienoic acid, 4-hydroxy- 3,5-dimethoxy,Tricyclo (2, 7) dec-8-ene-3-methanol, n-Hexanoic acid, 1, 3, 5-Triazine-2,4,6-triamine, cis-Vaccenic acid, 5-Hydroxymethylfurfural, 4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl, 9, 12-Octadecadienoic acid, 4-(2-Methoxyethyl)phenol, 3,7-Cyclodecadiene-1-methanol, Tricyclo[4.4.0.0(2,7)] dec-8-ene-3-methanol, Phenol, 4-(2-aminoethyl), 3,3'-Dimethoxy-4,4'-dihydroxystilbene, Propanoic acid, Ben-

Table 8. Compounds obtained by GC-MS analysis of *Balanites aegyptiaca* fruit extract

S. No.	Compound Name	Molecular Formula	Retention Time	Area %	Molecular weight
1	4-O-Methylmannose	$C_7H_{14}O_6$	21.427	41.04	194
2	3,4,5,6,7,9-Hexahydro-2H-xanthene-1,8-dione	$C_{24}H_{28}O_3$	23.613	27.45	364
3	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	$C_4H_9NO_5$	16.049	2.86	151
4	Acetic acid	$C_2H_4O_2$	1.949	2.85	60
5	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6	$C_6H_8O_4$	10.029	2.25	144
6	5-Hydroxymethylfurfural	$C_6H_6O_3$	11.6	1.79	126
7	9,12,15-Octadecatrienoic acid, methyl ester	$C_{19}H_{32}O_2$	24.889	1.49	292
8	Propylamine,N-[9-borabicyclo[3.3.1]non-9-yl	$C_{11}H_{22}BN$	18.86	1.21	179
9	Propanoicacid,3-(acetyloxy)-2-(hydroxymeth	$C_8H_{14}O_5$	12.725	1.18	190
10	3-Deoxy-d-mannoic lactone	$C_6H_{10}O_5$	18.65	1.15	162
11	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	24.434	1.03	294
12	4-Cyclopentene-1,3-dione	$C_5H_4O_2$	4.843	0.87	96
13	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	22.51	0.86	270
14	1,2,3-Propanetriol, 1-acetate	$C_5H_{10}O_4$	11.952	0.77	134
15	2-Propanone,1-hydroxy	$C_3H_6O_2$	2.272	0.69	74
16	1,4-Butanediol, diacetate	$C_8H_{14}O_4$	19.332	0.68	174
17	Glycerin	$C_3H_8O_3$	1.802	0.66	92
18	O-Methylisourea	$C_2H_6N_2O$	10.353	0.65	74
19	1,3,5-Triazine-2,4,6-triamine	$C_3H_6N_6$	8.756	0.61	126
20	2H-1,2-Oxazine, tetrahydro-2-methyl	$C_5H_{11}NO$	5.142	0.58	101
21	Butanoic acid, 3-methyl	$C_5H_{10}O_2$	4.32	0.48	102
22	Undecane	$C_{11}H_{24}$	7.03	0.41	156
23	2,5-Furandione, 3-methyl	$C_5H_4O_3$	5.903	0.39	112
24	3(2H)-Furanone,4-hydroxy-5-methyl	$C_5H_6O_3$	8.002	0.35	114
25	Methylacetoacetate	$C_5H_8O_3$	3.212	0.34	116
26	2H-Pyran-2,6(3H)-dione	$C_5H_4O_3$	6.961	0.32	112
27	2-Amino-1,3-propanediol	$C_3H_9NO_2$	1.86	0.29	91
28	Butanoic acid, 2-methyl	$C_5H_{10}O_2$	4.474	0.29	102
29	2(3H)-Furanone, 5-hexyldihydro-	$C_{10}H_{18}O_2$	11.413	0.26	170
30	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-on	$C_6H_8O_4$	6.678	0.25	144
31	3-Pentanone, 2-methyl	$C_6H_{12}O$	2.442	0.22	100
32	Propanoic acid, 2-methyl	$C_4H_8O_2$	3.083	0.22	88
33	2,5-Dimethylfuran-3,4(2H,5H)-dione	$C_6H_8O_3$	8.327	0.22	128
34	2-Furanmethanol	C5H6O2	4.391	0.21	98
35	Butanedioic acid, 2-hydroxy-2-methyl-,(S)-	$C_5H_8O_5$	13.751	0.21	148
36	2-Propenoic acid, methyl ester	$C_4H_6O_2$	3.012	0.2	86
37	N-Methoxy-N-methylamino-methyl-(S)-prolin	$C_9H_{18}N_2O_3$	16.749	0.2	202
38	Propanoic acid, 2-oxo-, methyl ester	$C_4H_6O_3$	2.715	0.19	102
39	alpha.-Amino-.gamma.-butyrolactone	$C_4H_7NO_2$	9.815	0.17	101
40	2-Pentanone	$C_5H_{10}O$	2.38	0.16	86
41	1-Cyclopentene-1-carboxylic acid	$C_6H_8O_2$	7.468	0.16	112
42	Carbonic acid, propargyl 3-pentyl ester	$C_9H_{14}O_3$	9.057	0.15	170
43	2-Octanol, acetate	$C_{10}H_{20}O_2$	11.063	0.15	172
44	2-Propanone, 1-(acetyloxy)	$C_5H_8O_3$	4.555	0.14	116
45	Propanoic acid, 2-hydroxy-, methyl ester	$C_4H_8O_3$	2.844	0.13	104
46	4-Heptanol, acetate	$C_9H_{18}O_2$	9.912	0.13	158
47	2,5-Furandione,3,4-dimethyl	$C_6H_6O_3$	7.684	0.11	126
48	4H-Pyran-4-one,3,5-dihydroxy-2-methyl	$C_6H_6O_4$	10.8	0.11	142

Table 8. Contd…...

49	1Decane,1-fluoro-	$C_{10}H_{21}F$	11.283	0.11	160
50	beta.-d-Ribopyranoside, methyl, 3-acetate	$C_8H_{14}O_6$	16.914	0.11	206
51	Methylfluoroacetate	$C_3H_5FO_2$	2.515	0.1	92
52	1,4-Dioxin, 2,3-dihydro	$C_4H_6O_2$	2.55	0.09	86
53	(S)-5-Hydroxymethyl-2[5H]-furanone	$C_5H_6O_3$	3.589	0.09	114
54	2-Cyclopenten-1-one, 2-hydroxy	$C_5H_6O_2$	5.642	0.09	98
55	6-Hydroxycyclooctane-1,2,5-trione	$C_8H_{10}O_4$	10.88	0.09	170
56	2(5H)-Furanone	$C_4H_4O_2$	5.35	0.08	84
57	Butyl 2-acetoxyacetate	$C_8H_{14}O_4$	17.29	0.07	174
58	Furfural	$C_5H_4O_2$	4.037	0.06	96
59	Propane-1,1-dioldiacetate	$C_7H_{12}O_4$	7.86	0.06	160
60	Methylacetoxyacetate	$C_5H_8O_4$	8.95	0.06	132
61	1,2-Butanediol	$C_4H_{10}O_2$	3.953	0.05	90
62	2-Furancarboxaldehyde, 5-methyl	$C_6H_6O_2$	6.31	0.05	110
63	3-Acetoxy-3-hydroxypropionic acid, methyl	$C_6H_{10}O_5$	9.517	0.02	162
64	3-Furanmethanol	$C_5H_6O_2$	3.869	0.01	98

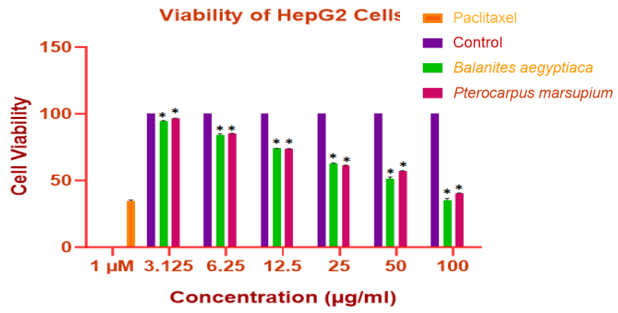

Fig. 2. *Cell viability against liver (HepG2) cells at different concentrations of plant extracts*

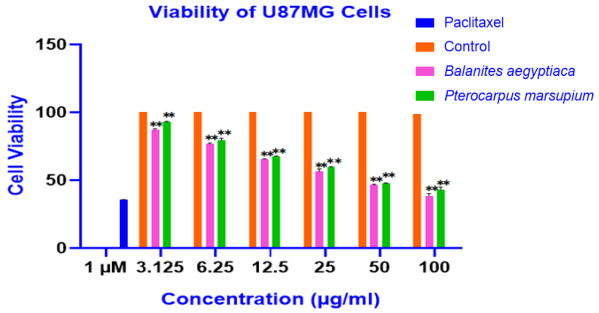

Fig. 3. *Cell viability against brain (U87MG) cells at different concentrations of plant extracts*

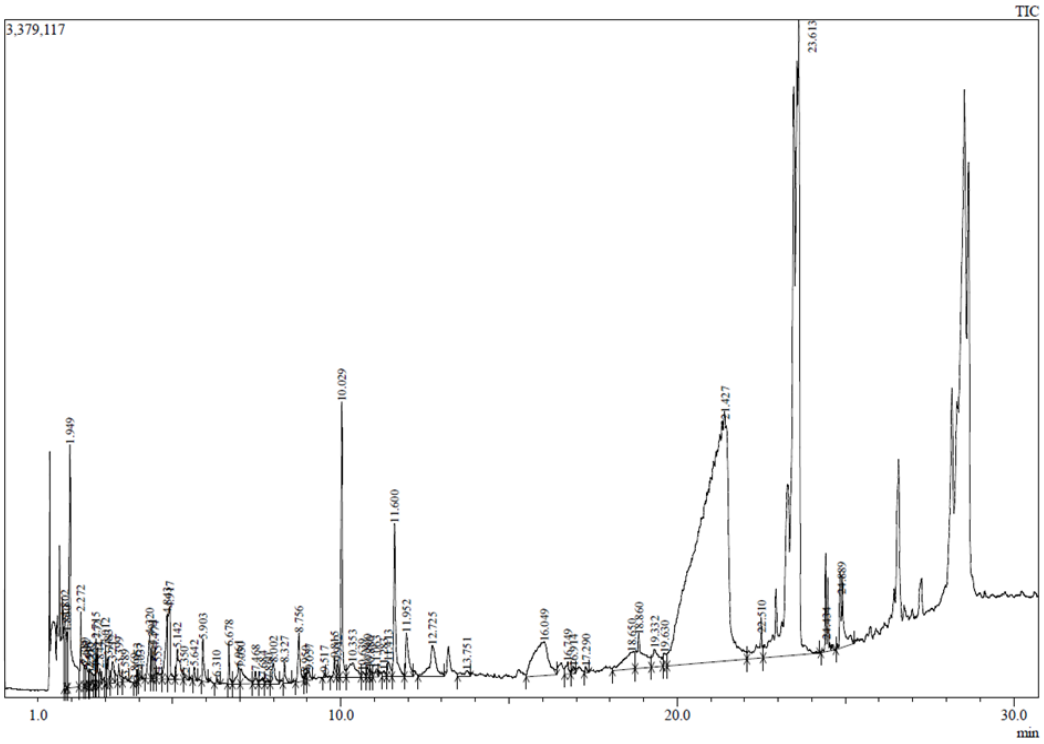

Fig. 4. *GC-MS Chromatogram for Balanites aegyptiaca fruit extract*

Table 9. Compounds obtained by GC-MS analysis of *Pterocarpus marsupium* heartwood extract

S. No.	Compound Name	Molecular Formula	Retention Time	Area %	Molecular weight
1	3,7-Cyclodecadiene-1-methanol	$C_{11}H_{24}$	22.396	17.06	156
2	Tricyclo[4.4.0.0(2,7)]dec-8-ene-3-methanol	$C_5H_4O_3$	22.274	16.35	112
3	Phenol, 4-(2-aminoethyl)-	$C_{10}H_{20}O_2$	28.385	9.84	172
4	2-Naphthalenemethanol, decahydro-	$C_5H_4O_2$	19.07	7.01	96
5	4-(2-Methoxyethyl)phenol	$C_6H_8O_4$	26.732	4.67	144
6	3,5-Dimethoxybenzyl alcohol	$C_5H_8O_3$	16.915	4.64	116
7	(3-Methoxyphenyl) methanol, n-butyl ether	$C_2H_6N_2O$	27.228	4.17	74
8	2-Naphthalenemethanol, 1,2,3,4,4a,5,6,7-octa	$C_5H_6O_3$	18.686	3.16	114
9	Benzoic acid, 4-hydroxy-	$C_4H_8O_2$	16.815	2.09	88
10	Benzaldehyde, 4-hydroxy-	$C_4H_6O_3$	14.247	2.04	102
11	(1R,4aR,7R,8aR)-7-(2-Hydroxypropan-2-yl)-1	$C_5H_6O_2$	21.396	1.69	98
12	2-Naphthalenemethanol, decahydro-.alpha.,.al	$C_6H_8O_2$	22.492	1.55	112
13	(1R,7S,E)-7-Isopropyl-4,10-dimethylenecyclo	C5H6O2	19.941	1.26	98
14	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-	$C_6H_8O_4$	22.096	1.11	144
15	Bicyclo[2.2.2]oct-5-ene, 2-methoxymethylene	$C_8H_{10}O_4$	27.758	1.05	170
16	7-Hydroxyflavanone	$C_6H_6O_4$	27.605	1	142
17	(E)-3,3'-Dimethoxy-4,4'-dihydroxystilbene	$C_{10}H_{18}O_2$	30.07	0.98	170
18	1,2-Bis(3,5-dimethoxyphenyl)-ethan-1,2-dione	$C_{10}H_{21}F$	28.71	0.97	160
19	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyp	$C_5H_8O_3$	20.145	0.88	116
20	2.beta.-HYDROXY-1.beta.,(4A).beta.-DIMET	$C_5H_4O_3$	21.808	0.87	112
21	n-Hexadecanoic acid	$C_5H_6O_3$	22.928	0.86	114
22	1-Naphthalenol, 1,2,3,4,4a,7,8,8a-octahydro-1	$C_5H_6O_2$	18.884	0.77	98
23	9,12-Octadecadienoic acid (Z,Z)-	$C_9H_{14}O_3$	24.83	0.77	170
24	N-Formyl-L-tyrosine	$C_5H_{10}O_2$	19.675	0.73	102
25	Acetic acid	$C_2H_4O_2$	1.89	0.7	60
26	cis-Vaccenic acid	$C_6H_{10}O_5$	24.887	0.64	162
27	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7	$C_5H_{10}O_2$	20.082	0.56	102
28	Glycerin	$C_3H_8O_3$	1.791	0.46	92
29	3,5-Dimethoxybenzaldehyde	$C_4H_6O_2$	15.404	0.46	86
30	(1R,7S,E)-7-Isopropyl-4,10-dimethylenecyclo	$C_5H_{11}NO$	20.73	0.45	101
31	2-Propanone, 1-hydroxy-	$C_2H_4O_2$	2.257	0.42	60
32	m-Guaiacol	$C_4H_6O_2$	11.64	0.41	86
33	Picrotoxin	$C_6H_8O_3$	24.411	0.39	128
34	2-Methyl-5-(2,6,6-trimethyl-cyclohex-1-enyl)-	$C_3H_6N_6$	24.475	0.37	126
35	Benzene, 1,3-dimethoxy-5-[(1E)-2-phenylethe	$C_9H_{18}O_2$	25.997	0.22	158
36	2-Propenoic acid, oxiranylmethyl ester	$C_3H_6O_2$	3.004	0.21	74
37	Benzofuran, 2,3-dihydro-	$C_3H_5FO_2$	11.515	0.21	92
38	Benzoic acid, 4-hydroxy-3,5-dimethoxy-, hydr	$C_5H_4O_2$	20.498	0.17	96
39	Hexanoic acid	$C_6H_{12}O$	6.704	0.14	100
40	Propanoic acid, 2-oxo-, methyl ester	$C_5H_{10}O$	3.393	0.12	86
41	1-Pentadecene	$C_4H_8O_3$	14.567	0.1	104

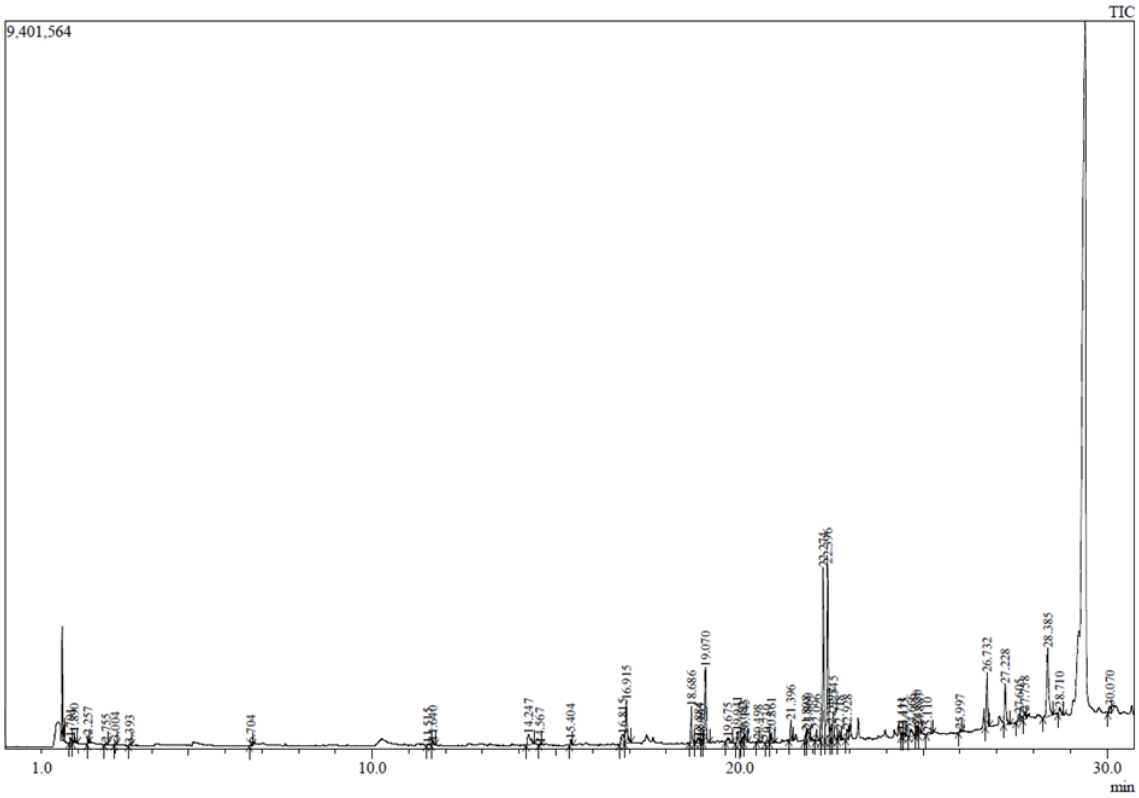

Fig. 5. *GC-MS Chromatogram for Pterocarpus marsupium heartwood*

zofuran, 2,3-dihydro, 1-Pentadecene and 7-Hydroxyflavanone are known for their potential antioxidant and anticancer activities.

Among the compounds having strong antioxidative potential, 4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl-, 9, 12-Octadecadienoic acid, 3,3'-Dimethoxy-4,4'-dihydroxystilbene, Propanoic acid, 7-Hydroxyflavanone, n-Hexadecanoic acid, 3,5-Dimethoxybenzyl alcohol, 9,12,15-Octadecatrienoic acid were studied (Kumar *et al*., 2010, Li and Seeram, 2010; Kostrzewa-Susłow and Janeczko, 2012; Yu *et al*., 2013; Reza *et al*., 2021).A study revealed that the fruits gathered from the desert region of Egypt exhibited notable components such as hexadecanoic acid, 9-octadecanoic acid, and β-sitosterol, which were prominently present (Ibrahim *et al*., 2022). In contrast, the present study focused on fruits sourced from the Indian arid region, where 4-O-methylmannose and 3,4,5,6,7,9-hexahydro-2H-xanthene-1,8-dione were the major phytoconstituents identified while hexadecanoic acid and octadecanoic acids were present minorly. This difference suggests that demography also plays a role in the availability of the phytoconstituents and their concentrations. Specifically, variations in environmental factors such as soil composition, climate, and altitude can significantly impact plant growth and the biochemical pathways that produce these compounds. The potential of several derivatives of 3,4,5,6,7,9-hexahydro-2H-xanthene-1,8-dione have been studied and have shown potent antioxidant and anticancer activities by *in vitro* and molecular docking studies (Retnosari *et al*., 2021; Ebadi *et al*., 2024). In another study, fractionation of the glucose–histidine MRPs (Maillard reaction products), which are produced by heating sugar-amino acid, was done to evaluate the antioxidant potential and it was found that the fraction which contained 4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl- had the strongest antioxidative activity as compared to other fractions (Yu *et al*., 2013). Another work evaluated the antioxidant potential of a flavonoid 7-Hydroxyflavanone and its biotransformed products. The DPPH assay revealed good antioxidative effects of all the compounds with 9.44 μM IC_{50} value for 7-Hydroxyflavanone (Kostrzewa-Susłow and Janeczko, 2012).

Stilbenes, the polyphenolic compounds produced by plants, are used in herbal formulations nowadays due to their antioxidant, antiproliferative and anti-inflammatory properties. Some of the most important stilbenes having medicinal properties are Resveratrol, pterostilbene and astringin (McCormack and McFadden, 2012; Ko *et al*., 2017; Al-Khayri *et al*., 2023). The GC-MS analysis of *P. marsupium* heartwood extract revealed the presence of 3,3'-Dimethoxy-4,4'-dihydroxystilbene. In a study, this compound was isolated from the butanol extract of maple syrup, which had antioxidant properties similar to that of a synthetic antioxidant reagent BHT (butylated hydroxytoluene) (Li and Seeram, 2010). Compounds such as 1,3,5-Triazine -2,4,6-triamine, 1,2,3-Propanetriol,n-Hexadecanoic acid, n-Hexanoic acid, 9,12-Octadecadienoic acid, 5-

Hydroxymethylfurfural are already being studied for their antiproliferative effects through *in vitro* and *in silico* studies (Kumar *et al*., 2010; Juneious and Rani, 2014; Abu Bakar el al., 2015; Ravi and Krishnan, 2017; Jose *et al*., 2018; Reza *et al*., 2021). n- Hexadecanoic acid extracted from the crude extract of *Kigelia pinnata* leaves inhibited HCT-116 cancer cell proliferation with an IC_{50} value of 0.8µg/ml. The docking studies revealed that the activity could be due to the higher affinity of the isolated compound towards DNA Topoisomerase I, which inhibits cancer cell proliferation (Ravi and Krishnan, 2017).

In another study, a compound tricaproin obtained from the plant *Simarouba glauca*, inhibited the proliferation of colorectal cancer cells by inducing apoptosis. Further fractionation and characterization of the compound revealed that n-hexanoic acid was the major constituent responsible for this activity. The *in silico* study showed the mechanism of apoptosis induction and antiproliferation was due to the reduction of Class-I Histone deacetylases by the action of tricaproin and n-hexanoic acid (Jose *et al*., 2018). The anticancer potential of different parts of fruits of *Garcinia dulcis* was evaluated against HepG2 cells, which revealed the lowest IC_{50} value as 38.33±3.51 µg/ml of the flesh extract. The GC-MS results revealed 5-Hydroxymethylfurfural as the major component responsible for the anticancer and apoptotic activity of the extract (Abu Bakar *et al*., 2015).

The studies mentioned above indicate that the antioxidant and antiproliferative properties of crude extracts from *B. aegyptiaca* and *P. marsupium* can be attributed to the active phytoconstituents present, including phenols, flavonoids, fatty acids, esters, sugars, and other significant secondary metabolites found in the methanolic extracts.

Conclusion

In conclusion, the present study highlights the significant potential of the methanolic extracts of *B. aegyptiaca* and *P. marsupium* in providing antioxidant and anticancer properties, primarily due to the diverse array of phytoconstituents identified. The presence of various secondary metabolites, including alkaloids, phenols, flavonoids, and terpenoids, not only supports the traditional use of these plants in herbal medicine but also underscores their relevance in modern therapeutic contexts.The observed total phenolic content and total flavonoid content indicate a robust capacity for combating oxidative stress, which is a crucial factor in the pathogenesis of many chronic diseases, including cancer. Furthermore, the IC_{50} values obtained in present assays demonstrate the extracts' effectiveness in inhibiting the proliferation of hepatoma (HepG2) and glioblastoma (U87MG) cancer cells, highlighting their potential as alternative or complementary treatments in cancer therapy. Notably, specific compounds such as 4-O-methylmannose, 3,4,5,6,7,9-hexahydro-2H-xanthene-1,8-dione,4H-Pyran-4-one and 3,3'-Dimethoxy-4,4'-dihydroxystilbene exhibited promising antioxidant and antiproliferative activities, indicating that these phytochemicals could serve as leads for the development of novel therapeutic agents. This study thus highlights the need for further research into the mechanisms and clinical efficacy of these compounds. It underscores the importance of exploring plant-derived compounds for anticancer therapies and calls for comprehensive investigations into their phytochemical profiles across regions to understand their medicinal potential better and promote sustainable use in healthcare.

Conflict of interest

The authors declare that they have no conflict of interest.

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