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Green Synthesis of AgO Nanoparticles and Its Biological Applications

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KEYWORDS

Nanoparticles, GCMS and NMR, morphologies

Abstract

Simple green synthesis was used to synthesis silver oxide nanoparticles. The prepared materials were characterized different analytical techniques such as XRD, SEM, FT-IR, GCMS and NMR studies. AgO have a crystalline of 29.2 nm, respectively. Through scanning electron microscopy (SEM), surface morphologies of AgO nanoparticles were found to exhibit irregular structures and a spherical, agglomerated matrix. The prepared materials were utilized to the anti-cancer agent of human body and the materials were cached the cancer infected cells up to 90%.

Introduction

With a broad variety of applications in the disciplines of health and medicine, nanobiotechnology is an advanced technology in biological activity for the manufacture of nanosized particles. Because of their distinct structural[1], biological[2] and optical characteristics, nanoparticles can be used as masterminds in a variety of applications, including drug delivery[3], biosensors[4], regulating aberrant molecular mechanisms in the body, limiting the growth of infectious agents, and controlling the rate of abnormal cell proliferation. Numerous metal-based nanoparticles exist, including those made of gold, silver, zinc, iron, titanium, magnesium, and alginate. One of the most fatal illnesses, cancer claims the lives of millions of individuals every year globally. Usually originating from the cervix, the Human Papilloma Virus is the cause of cervical cancer. The fourth most prevalent cause of cancer and, ultimately, death among women is cervical cancer. Warts develop around the vaginal regions as a consequence of this malignancy. But if the right care is provided and caught early, it may be prevented and cured. In this work, HeLa cell lines were used to assess the effects of green synthesised nanoparticles on cytotoxicity and apoptosis (a process that causes nuclear disintegration). Silver nanoparticles' distinct morphological features may help them get past obstacles and into the target cells.

Materials and method

This work examined the pace at which the development of cancer cells is inhibited, as well as the generation of reactive oxygen species, malondialdehyde, and glutathione storage in MCF7 cells. The 48 hours, cancer cells were exposed to nanoparticles. Silver nanoparticles both slowed MCF-7's rate of development.

Synthesis of silver oxide nanoparticles

Silver oxide nanoparticles were synthesised via the eco-friendly green synthesis method. The 20 mg of plant extract was washed with water and ethanol. The mixture was added to the beaker and 70 mL of ethanol and 30 mL of water were added. In addition to that, 3.2 g of silver nitrate was added to the beaker. The reaction mixture was stirred at room temperature for 10 mins and the temperature was raised to 90ºC to be maintained for one hour[5]. Finally, the green-coloured precipitate was formed, the precipitate was filtered to get silver hydroxide nanoparticles. The product of silver hydroxide was calcinated at 450ºC for 5 hours to get the final product of the silver oxide nanoparticles.

XRD analysis

XRD analysis, which determined the crystalline size and geometry of the prepared material, was carried out using the Debye-Scherrer equation. The corresponding planes 111, 200, 220 and 311 are found to be 2θ of 38, 45, 67 and 78, respectively. The sharp peaks indicate the prepared materials were presented in a well-crystal nature with high purity. The crystalline size of the prepared AgO was found to be 29.2 nm. AgO nanoparticles are acting as good anti-cancer agents due to their lower crystalline size.

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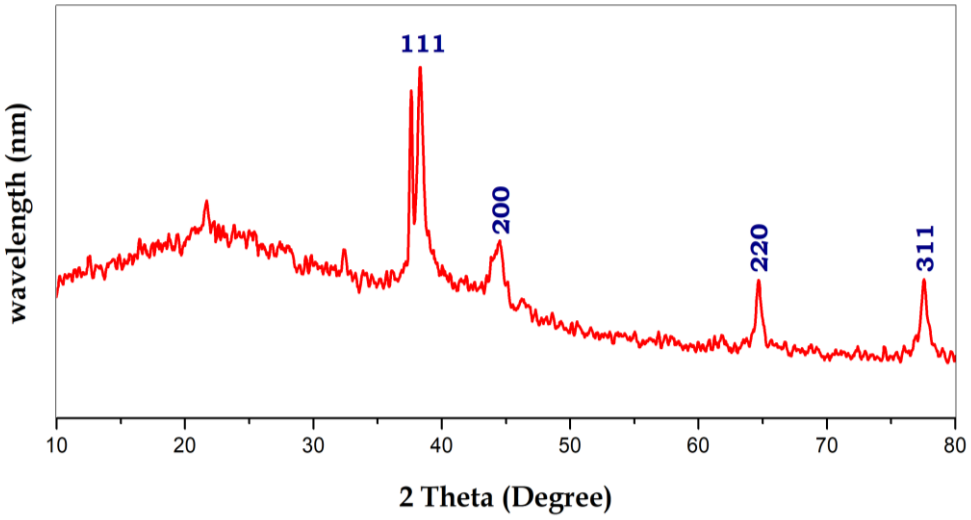

Fig.1.XRD analysis of AgO nanoparticles

Functional group analysis

Functional group analysis is used to conform the different functional groups present in our prepared material. Stretching vibration mode of M-O, present in the 824.6 and 1042cm^{-1} respectively. Stretching mode of C=O, C-O was presented in 1341 and 1612 cm^{-1} [6]and the vibration peaks of H-O-H was appeared at 3344 cm^{-1}[7]. FT-IR conforms the metal -oxygen stretching vibration mode are presented in below 1000 cm^{-1}, it is explained the purity of the prepared materials.

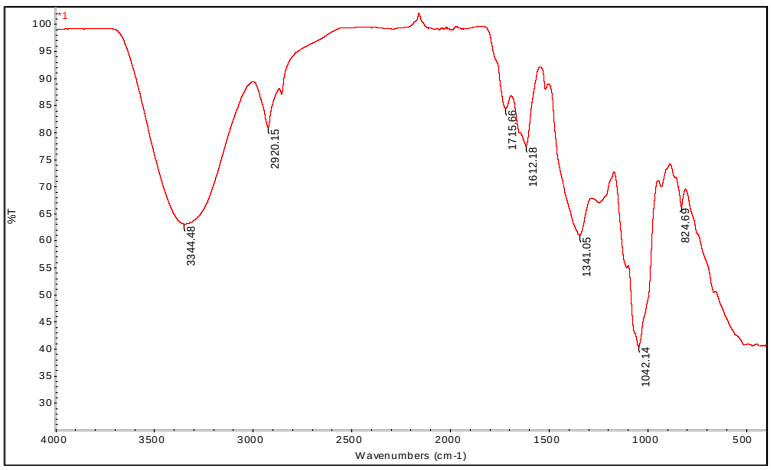

Fig.2.FT-IR analysis of AgO nanoparticles

NMR analysis

^{1}H and ^{13}C NMR analysis

A Bruker Avance DRX 500 was used to record NMR spectra. Every NMR spectrum was recorded at 303 K in DMSO-d6. The solvent resonance was used as a reference point for the ^{1}H and ^{13}C NMR chemical shifts (δ in ppm) for DMSO-d6 (2.50 (^{1}H) and 39.50 (^{13}C) respectively. The distinct 1H and 13C resonances were identified using gradient-selected COSY, gs-HSQC, gs-HMBC, and 2D NMR studies. The ^{1}H-^{13}C HMBC experiment was calibrated for long-range coupling of 500.13 and 125.758 MHz, and the gradient ratio was 30:18:24 G cm^{-1}[8]

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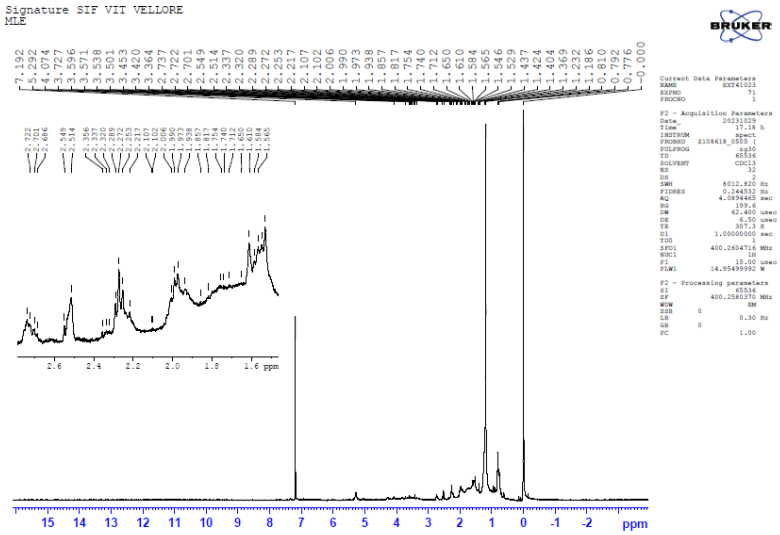

Fig.3.[1]H NMR analysis of AgO nanoparticles

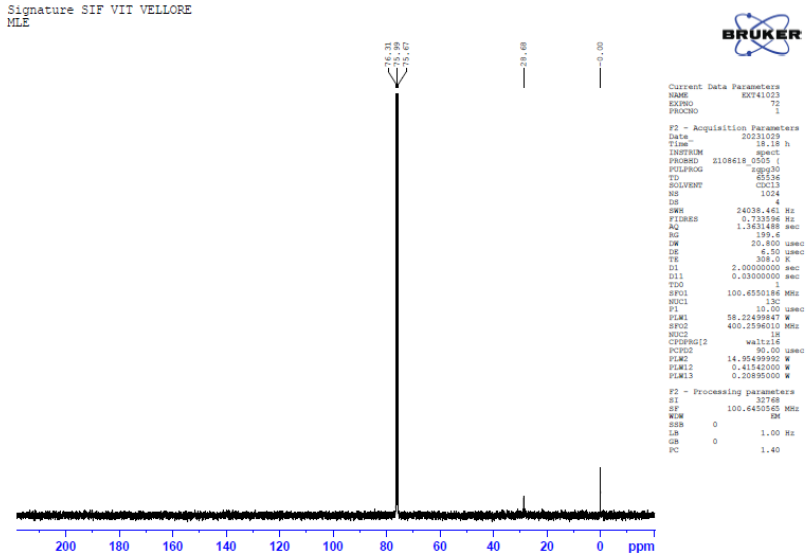

Fig.4.[13]C NMR analysis of AgO nanoparticles

Table 1. [1]H, [13]C NMR spectral data of compound 2 (β-sitosterol)

Carbon number	Chemical shifts (δ)	
	[1]H NMR data	**[13]C NMR data**
1	-	37.250
2	-	31.652
3	3.52 (1H,m)	71.818
4	-	42.294
5	-	140.752
6	5.35 (1H,m)	121.713
7	-	31.904
8	-	31.905
9	-	50.135

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10	-	36.501
11	-	21.075
12	-	39.773
13	-	42.294
14	-	56.766
15	-	23.068
16	-	29.254
17	-	56.060
18	0.66(3H,s)	11.847
19	1.1(3H,s)	19.451
20	-	36.135
21	0.91(3H,d.J-6.4Hz)	18.771
22	-	33.952
23	-	23.067
24	-	50.134
25	-	31.912
26	0.82(3H,d,J=6.8Hz)	19.810
27	0.69(3H,d,J=6.9Hz)	19.031
28	0.85(3H,t,J=7.8)	28.236
29	-	11.965

Anticancer Activity

Cancer is a multifaceted illness that often has a broad spectrum of increasingly severe consequences at the cellular and molecular levels. Therefore, it doesn't appear plausible that chemoprevention adheres to rigid guidelines and specifications.

Cell culture

The human gastric carcinoma cell line Kato-III, the hepatocellular carcinoma cell line Hep G2, the lung adenocarcinoma epithelial cell line SHP-77, and the various mammalian breast cancer cell lines MCF-7 were all cultured in minimum essential medium (MEM) supplemented with EDTA, sodium bicarbonate, and foetal calf serum. The cultures were then kept in a humidified environment at 37°C and 5% CO_2. Every two days, the culture medium was swapped out. To more accurately resemble how plant extracts might effect human cancer cells, all of the cell lines employed were of the human species. Generally, cells were cultivated at 37˚C in a humidified environment of 5% CO_2/95% air in 75 cm^2 tissue culture (T-75) flasks with 10 mL of the appropriate media[9]. Weekly cell passages and fortnightly medium replacements were performed.

Subculture

Aspiration was used to remove the medium, and adherent cells were then cleaned with 5 millilitres of Calcium and Magnesium Free solution (CMFS) to get rid of any remaining media that contains FCS, a trypsin inhibitor. To dissolve the extracellular matrix and lift the cells, each flask received around 1 millilitre of a 1X trypsin-EDTA solution in CMFS. For a maximum of 10 minutes, they were incubated at 37˚C, and during that time, the cells were observed under a microscope for indications of separation[10]. The addition of 9 mL of suitable medium containing FCS inactivated the trypsin. Depending on the cell line and the time when the cells were needed, subculturing ratios ranging from 1/5 to 1/50 were used.

Cell viability assay

The MTT test quantifies the number of live cells by seeing how metabolically active cells change the yellow tetrazolium salt (MTT) into purple formazan crystals (Mosmann, 1983). After plating cells at a density of 2×105 mL^{-1} per well in 100 µL of Rosewell Park Memorial Institute 1640 (RPMI 1640), the cells were incubated in a CO_2 incubator at 37°C and 5% CO_2 for a full day. Each well received an ethanolic extract of Butea monosperma dissolved in DMSO after 24 hours, and the wells were then incubated for 48 hours[11]. The identical quantity of DMSO was given to the control groups. A positive control was provided by tamoxifen. The cells were incubated at 37°C with 5% CO_2 for 24 to 48 hours. Subsequently, each well

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was supplemented with 100 µL of MTT ([3- (4, 5-dimethylthiazol-yl)-2, 5-diphenyl tetrazolium bromide]) solution (0.5 mg/mL in Dulbecco's modified eagle's medium) and incubated for three hours. The capacity of live cells to convert the yellow dye MTT into a blue formazan product was used to measure the growth of tumoral cells. MTT reduction's formazan product was dissolved in DMSO. After removing the medium, each well was filled with 100 µL of DMSO to dissolve the MTT metabolic product. After that, the plate was shaken for five minutes at 150 rpm, and the optical density at 570 nm was recorded. In order to get the IC50 values, percentage inhibitions [100 - (absorbance of test wells/absorbance of control wells) 100] were computed and plotted against the concentrations.

RESULTS

Today, butea monosperma is utilised as medicine by the largest tribal tribe in this area. Natural treatment for numerous human disorders refined using plant components has attracted great interest. In rural areas around the globe, the use of herbal remedies for medicinal purposes is widespread. Cancer is a disease characterised by a population of cells that grow and proliferate uncontrollably, infiltrate and destroy nearby tissues, and may metastasize—spread to distant anatomic areas—to other places. Although cancer may strike anybody at any age, the risk increases with age for the most prevalent types. 17% of fatalities are related to cancer[12]. Plants have been integral to the traditional Indian therapeutic system, particularly in relation to cancer prevention. In order to prevent and treat cancer disorders, a number of herbs and herbal products have been suggested. The ethanolic extract of Butea monosperma possesses antioxidant activity, which may help prevent the development of cancer disorders, as the current research has clearly shown.

Physicochemical Analysis

Table 1 displays the findings of the proximate analysis of Butea monosperma whole plant powder. The percentage of air-dried material is used to express the average values. The extract produced by the serial extraction procedure was subjected to phytochemical analysis, and the presence of chemicals in each extract was reported. We calculated the total ash values, water soluble ash content, acid insoluble ash content, and the percentage of loss on drying. In all, 6.48% of the ash was water soluble, 3.15% was acid insoluble, and 1.22% was water insoluble[13]. Ash values show the drug's purity, and loss on drying value shows that there is no chance of bacterial or fungal development or contamination. The water extract had a pH of 5.98 and a melting point of 110°C, respectively[13].

Any crude medication can be extracted using a specific solvent to produce a solution with a variety of phytoconstituents based on the results of extractive values (Table 2). The type of medication and solvent utilised determine the compositions of these phytoconstituents. It is possible to obtain preliminary data regarding a drug's quality by using a single solvent. Additionally, extractive value provides information on the drug's quality. The extraction value of Butea monosperma whole plant powder was highest in ethanol (40.22%), lowest in water extract (18.86%), and then highest in methanol (35.74%), ethyl acetate (32.29%), hexane (26.35%), petroleum ether (24.41%), and acetone (20.27%). It was found that ethanol and methanol were the most effective solvents for extractive values. The extractive values (Fig. 3) indicated whether polar or nonpolar chemicals were present[14].

Table 2. Proximate analysis of *Butea monosperma*

S. No	Parameters	Values (%w/w)
1	Total Ash value	6.48 ± 0.14
2	Water soluble Ash value	3.15 ± 0.19
3	Acid insoluble Ash value	1.22 ± 0.35
4	Ethyl acetate soluble extractive	32.29 ± 0.27
5	Petroleum ether soluble extractive	24.41 ± 0.12
6	Hexane soluble extractive	26.35 ± 0.21
7	Methanol soluble extractive	35.74 ± 0.14

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8	Acetone soluble extractive	20.27 ± 0.18
9	Ethanol soluble extractive	40.22 ± 0.52
10	Water extract	18.86 ± 0.31
11	pH of extract	5.98 ± 0.13
12	Melting point water extract	110 °C
13	Loss On Drying	5.41

Solubility test

The solubility of Butea monosperma plant powder was assessed in 10 distinct solvents with varying polarity. In solvents such as acetone, dimethylformamide (DMF), petroleum ether, distilled water, and toluene, the extract was only moderately soluble. In contrast, it was extremely soluble in ethanol, methanol, ethyl acetate, dimethyl sulphoxide (DMSO), and hexane[14].

Table 3. Solubility of *Butea monosperma* in different solvents

S. No	Solvents	Solubility (mg/ml)
1	Acetone	19.12 ± 0.15
2	Dimethylformamide (DMF)	21.10 ± 0.34
3	Dimethylsulphoxide (DMSO)	27.12 ± 0.19
4	Distilled water	15.50 ± 0.28
5	Ethyl acetate	31.57 ± 0.64
6	Hexane	26.34 ± 0.12
7	Methanol	34.15 ± 0.41
8	Petroleum ether	20.71 ± 0.27
9	Ethanol	38.21 ± 0.19
10	Chloroform	20.21 ± 0.23

Mineral Element Composition

Table 4 displays the mineral element compositions of Butea monosperma. Iron (Fe), magnesium (Mg), copper (Cu), zinc (Zn), manganese (Mn), calcium (Ca), sodium (Na), potassium (K), and phosphorus (P) were the mineral elements identified.

Iron (Fe)

Butea monospermic had an iron content of 110.5 ± 0.12 ppm. Iron is necessary for hemoglobin to carry oxygen and is also involved in the metabolism of energy. Anaemia is the result of an iron deficiency. Based on the results, this plant may help diabetic patients who are iron deficient by reducing their anaemia.

Copper (Cu)

The copper content of Butea monospermic was measured and found to be 4.15 ± 0.54 ppm. Because it is necessary for the correct utilization of iron and for the creation of cytochrome oxidase, which is composed of both copper and iron, copper is an essential mineral in the diet. The acquired value is deemed suitable and safe for the human body.

Zinc (Zn)

Butea monospermic has a zinc level of 35.2 ± 0.35 ppm. Certain enzymes, including as carboxypeptidase, alkaline phosphatase, and dehydrogenase, need zinc in order to function. Organic compounds containing zinc are used as antifungal and astringent agents.

Magnesium (Mg)

Butea monospermic had 98.2 ± 0.14 ppm of magnesium. The enzyme reactions involved in the metabolism of ingested carbohydrates need magnesium. α-amylases, lactase, sucrase, and maltase are a few examples of these enzymes. In the electrical breakdown of nutrients and other elements within the cells, it is crucial.

Calcium (Ca)

The calcium content of *Butea monosperma* was found to be high 925 ± 1.08 ppm. Calcium is necessary for the strong bones and teeth. It is relatively high in cereals, nuts and vegetable.

Manganese (Mn)

Similar to iron and zinc, manganese is not very harmful to mammals. Mn traces are necessary for optimal

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health. Mn-containing compounds are utilised as tonics and as anaemia treatments. Manganese is used by the enzymes transketolase and ascorbate. The manganese concentration of Butea monosperma in this research was 42.2 ppm.

Potassium (K)

Butea monosperma had a potassium level of 481 ± 1.13 ppm. Potassium controls nerve function and is crucial for maintaining the body's acid-base, water-and electrolyte balance, and water balance. Butea monosperma has a high potassium content, which suggests that eating it will help the body maintain its acid-base balance and osmotic pressure.

Sodium (Na)

The sodium content of *Butea monosperma* was 725 ± 1.27 ppm. Sodium is the principal extracellular cation and is used for acid–base balance and some osmo-regulation in the body fluid.

Phosphorus (P)

The phosphorus content of *Butea monosperma* was 228 ± 1.09 ppm. Phosphorus is a constituent of bone and teeth, nucleoprotein, phospholipids, enzymes and high energy compounds.

Table 4. Inorganic element compositions of *Butea monosperma*

S. No	Elements	Concentration (ppm)
1	Iron (Fe)	110.5 ± 0.12
2	Zinc (Zn)	35.2 ± 0.35
3	Copper (Cu)	4.15 ± 0.54
4	Magnesium (Mg)	98.2 ± 0.14
5	Calcium (Ca)	925 ± 1.08
6	Manganese (Mn)	42.2 ± 0.32
7	Potassium (K)	481 ± 1.13
8	Sodium (Na)	725 ± 1.27
9	Phosphorus (P)	228 ± 1.09

Qualitative Phytochemical Analysis

The whole plant powder of Butea monosperma was refluxed sequentially in an equipment utilising a distilled ethanol solvent system based on polarity. The secondary metabolites were present in significant amounts in the ethanolic extracts of Butea monosperma[15]. The analysis conducted on the plant samples detected the existence of bioactive compounds with therapeutic properties. The phytochemical properties of the Butea monosperma findings were outlined. Qualitative phytochemical analysis was conducted on ethanolic extracts of selected medicinal plants of Butea monosperma to screen for alkaloids, flavonoids, cardiac glycosides, phlobotannins, steroids, tannins, triterpenes, saponins, fixed oil & lipids, gum & mucilage, carbohydrates, proteins, and amino acids. Analysis of the extracts revealed the existence of alkaloids, flavonoids, cardiac glycosides, phlobotannins, steroids, tannins, triterpenes, saponins, carbohydrate, protein, and amino acids in the ethanolic extracts of Butea monosperma. Table 5 displays the findings of qualitative phytochemical analysis. The ethanolic extract of Butea monosperma powder has a significant concentration of steroids and flavonoids[16]. Alkaloids, tannins, saponins, triterpenes, cardiac glycosides, phlobotannins, carbohydrates, proteins, and amino acids were moderately prevalent. The ethanolic extract of Butea monosperma did not include fixed oil, lipids, gum, or mucilage.

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Table 5. Qualitative Phytochemical analysis of *Butea monosperma*

S. No	Name of Test	Test applied / Reagent used	Observation
1	Alkaloids	Mayer's Wagner's Hagner's Dragndorff's test	+ ++ ++ ++
2	Flavanoids	Shinoda test Alkaline reagent test	++ +++
3	Cardiac glycosides	Keller-kilianni test	+
4	Phlobotannins	HCl Test	+
5	Steroids	Libermann-Burchard's	+++
6	Tannins	$FeCl_3$ test	++
7	Triterpenes	H_2SO_4 test	+
8	Saponins	Frothing test	++
9	Fixed oils & fats	Spot test	-
10	Gum & mucilage	Alcoholic Precipitation	-
11	Protein and Amino acids	Millons test Ninhydrin test	++ ++
12	Carbohydrate	Molisch`s test	++

Quantitative Phytochemicals Analysis

Phytochemicals are natural biochemical molecules that plants produce to defend themselves against oxidation, insect damage, and other environmental threats. Phytochemicals provide distinct colour, taste, aroma, and consistency. Epidemiological studies show that populations that consume high quantities of plant-derived foods have a low incidence rate of many illnesses (Table 6)[17].

Table 6. Quantitative Phytochemical Analysis of *Butea monosperma*

S. No	Parameters	Values (mg/gm)
1	Phenols	**39.2 ± 1.02**
2	Flavonoids	**32.8 ± 1.18**
3	Tannins	**25.1 ± 1.37**
4	Alkaloids	**11.2 ± 1.32**
5	Steroids	**43.2 ± 1.17**
6	Terpenoids	**12.5 ± 1.05**
7	Glycosides	**5.75 ± 0.22**
8	Ascorbic acid	**8.73 ± 0.41**
9	Saponins	**10.8 ± 0.65**

Phenols

The Butea monosperma ethanolic whole extract had a total phenolic content of 39.2 ± 1.02 mg/g as shown in Table 5 (Fig. 6). Phenolic acids are plant compounds found extensively in many plants. Phenolic chemicals are crucial for plant development and reproduction, since they are synthesised in reaction to protect wounded plants from pathogens. In recent years, the significance of the antioxidant properties of phenolic compounds and their potential use as natural antioxidants in processed foods has greatly increased.

Anticancer Activity of *Butea monosperma*

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Cancer occurs when there is a disruption in the equilibrium between cell growth and cell death, resulting in abnormal cell growth and tumour formation. The research examined the anticancer properties of the ethanolic whole plant extract of Butea monosperma by conducting an MTT experiment on four human cancer cell lines: HepG2, MCF7, SHP-77, and Kato-III. Succinate dehydrogenase, a mitochondrial enzyme, breaks down the tetrazolium ring in live cells, converting MTT into an insoluble purple formazan. The quantity of formazan generated is directly linked to the quantity of viable cells[18]. 5-fluorouracil, a versatile anticancer drug, often used in treating several types of cancer, was utilised as a positive control in this investigation.

Four cell lines from distinct tissues were assessed using samples. The cell lines used were MCF-7 from breast cancer, HepG-2 from liver, SHP-77 from lung, and Kato-III from gastric origin. All extract samples exhibited cytotoxic effects ranging from 0% to 88.8% across different cell lines. The highest cytotoxic activity was seen in SHP 77, with growth inhibition rates exceeding 85.7% and 84.3% in Hep G2 and Kato-III cells, respectively. Significant growth suppression was achieved using the whole plant extract of Butea monosperma against all cell lines examined[19].

Table 7. Anticancer activity of MCF-7 cell line of ethanolic extracts of *Butea monosperma*

S. No	Concentration (mg/ml)	% of Cell viability	% of Cytotoxicity
1	**Control**	**100**	**0**
2	**7.9**	**82.3**	**18.3**
3	15 .6	74.5	25.5
4	32.2	62.2	37.8
5	**51.5 (LC_{50})**	**48.9**	**50.8**
6	62.5	45.6	54.4
7	125	38.2	61.8
7	250	32.9	67.3
8	500	26.5	73.5
10	**1000**	**15.4**	**84.9**
11	**Positive control**	**10.8**	**89.5**

Table 8. Anticancer activity of MCF-7 cell line of β-sitosterol

S. No	Concentration (mg/ml)	% of Cell viability	% of Cytotoxicity
1	**Control**	**100**	**0**
2	0.78	78.2	21.8
3	1.56	67.4	32.6
4	3.12	57.8	42.2
5	**4.71 (LC_{50})**	**50.2**	**49.8**
6	6.25	43.1	56.9
7	12.5	38.5	61.5
7	25	30.9	69.1
8	50	23.5	76.5
10	**100**	**18.9**	**81.1**
11	**Positive control**	**10.5**	**89.5**

Conclusion

The auction determined that the conventional system of medicine is still commonly used for a variety of reasons. The expanding population, limited availability of medications, adverse effects of many allopathic therapies, and rising resistance to present treatments have highlighted the need of using plant materials as a medicinal resource for humans. This review provides thorough information on the phytochemicals and biological activities of plant extracts, which might

provide significant evidence supporting the plant's usage in various medications. In the near future, Butea monosperma might be used more extensively as a source of beneficial phytochemical substances and could have a significant impact on contemporary medicine. Clinical investigations may be done to further demonstrate the cytotoxic effects of β-sitosterol and ethanolic extract of Butea monosperma on cancer patients. The current investigation suggests that the chosen ethanolic extract of Butea monosperma has antibacterial activities against bacterial and fungal infections. The research demonstrated that the extract of Butea monosperma had significant antibacterial activity against both gram-positive and gram-negative human infections. The research demonstrates that the ethanolic extract of Butea monosperma has significant anti-cancer activity in cell lines. The presence of β-sitosterol phytocompounds is likely responsible for the anti-cancer effect.

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