ECO-FRIENDLY SYNTHESIS OF GOLD NANO PARTICLE (AuNP) AND ITS STUDIES OF PHARMACOLOGICAL ACTIVITIES USING AQUEOUS EXTRACT OF ALOE BARBADENSIS

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ABSTRACT

The present study aims towards the Eco friendly (Green) Synthesis of gold nano particle (AuNPs) and study their Pharmacological activity. Gold has good ductlity, moleculer recognition properties and good biocompatibility. At present gold is being used in many fields. GNP physical and chemincal properties vary with their size in nanometers. The surface area of a nanosized gold surface has a special effect. Therefore gold nanoparticle can directly and indirectly, give rise to different biological activities. The sulfided Gold substances have a strong Chemical reactivity and are easy to combine with Sulfhydryl groups, hence nanogold is often used in biomedical testing, disease, diagnosis and gene detection. Nanogold binds to proteins as antibodies, enzymes or cytokines. In fact, Scientist use nonogold to bind Special antibodies, as a tool for targeting Cancer cells. Gold name particles are also directly cytotoxic to cancer cells. For diseases caused by inflammation and oxidative damage, AuNPs also have antioxidant and anti-inflamatory effects. Based on these unique properties, AuNPs have become the most widely studied metal nanomaterials. Many recent Studies have further demonstrated that AuNPS are beneficial for humans, due to their functional Pharmacological properties in variety of diseases. In this study, we report a novel method of gold nonoparticle (AuNPs) synthesis using aqueous extract of leaves of Aloe barbadensis miller (Aloe vera). The phytochemicals present in the plant extract (PE) act as an effective reducing and Capping agent to synthesize AuNPs. The use of PE for green sythesis are cost effective and eco-friendly. The synthetized AuNPs were Characterised by Specto photometry, X-ray diffraction (XRD) and Fourier transform infrared (FTIR) spectroscopy.The XRD patterns showed peaks at (111), (200), (220) which exhibited preferential orientation of the AuNPs as face Centred cubic crystal. FTIR measurements Confirmed the Coating of phonilic compounds on the AuNPs indicating a possible role of biomolecules for the Capping and efficient Stabilization of the AuNPs. UV- visible Spectra also showed peak at 540 nm & characteristics of AuNPS.

KEY WORD:- green synthesis, AuNP, Aloe barbadensis (aloe vera) , phytochemicals,cytotoxic.

INTRODUCTION

In recent years, metallic nanoparticles, particularly gold and silver nanoparticles were used as theranostic agents for diagnosis and treatment of various disorders like diabetes, cancer, Parkinson's, Alzheimer's, HIV/AIDS, arthritis, hepatitis, cirrhosis, spinal cord injury, tuberculosis and cardiovascular diseases (CVD) due to their unique optoelectronic and physicochemical properties, and convenience of synthesis, uniqueness and surface modification in the nanoscale

range. Gold nanoparticles (AuNPs) gained interest for their various beneficial therapeutic, diagnostic and numerous technological applications. Nanoparticles are of immense interest due to their extremely small size and high surface area to volume ratio, which lead to both chemical and physical differences in their properties (e.g. mechanical and biological properties, catalytic activity, thermal and electrical conductivity, optical absorption and melting point) compared to bulk [1, 2]. Metallic nanoparticles exhibit various size and shape-dependent optical properties, which are useful in various biomedical applications like the imaging of specific target cells and tissues, drug delivery, bio-sensing and catalysts to optics, etc [3]. Furthermore, there were diverse applications of nano particles as antimicrobial agents, computer transistors, chemical sensors, and magnetic nanoparticles as a contrasting agent in magnetic resonance imaging [4].

In particular, AuNPs have proved to be highly versatile for deep-tissue imaging, the detection of heavy metals [5], as contaminants in the environment and can be favorably incorporated into clinical applications [6,7]. Physical methods (sonication, thermal, microwave) facilitate various chemical methods (sodium citrate and other reducing agents) to synthesize AuNPs [8]. In such procedures, traces of unreacted reagents remain in the gold colloidal solution rendering the AuNPs inappropriate for biological application. Since the first evidence of the capability of living systems to reduce metal ions like Au, Pt,Ag and Fe to zero valent form, interest has developed in the study of the bio-reduction potential of, plants and other green non-toxic reducing agents [9-12].

Physical and chemical methods of AuNP synthesis of involve the application of harsh reducing and stabilizing agents. To circumvent such issues, alternative formulations were developed to prepare AuNPs which have resulted in the exploration of herbal methods. Green synthesis methods rely on biological resources (fungi, yeast, bacteria, viruses, algae, plants) that are capable of replacing synthetic chemical reducing agents [13-15]. The green synthesis of GNPs offers advantages including increased biocompatibility. The mixture of GNPs and green reductants may result in synergistic biological activities. Various plant parts can be exploited as capping and stabilizing agents in the green synthesis of AuNPs [16, 17]. The ethno-medicinal property of aloe barbadensis extract prompted us to carry out the present investigation to synthesize AuNPs in an eco-friendly manner.

EXPERIMENTAL

1.Preparation of Alovera plant extract

Fresh leaf of Alovera plant was washed and peeled out. It was washed throughly and dipped in 100 ml double-distilled water & boiled for 30 minutes. It was cooled at room temperature and aqueous core extract (ACE) was filtered through whatman 42 filter paper and stored at 4ºc.

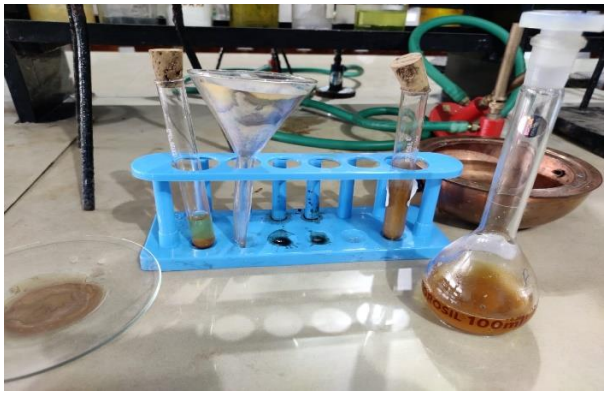
2.Preparation of 10 mM $HAuCl_4$ (aq) at room temperature under static Condition.

About 1 gm of $HAuCl_4$ was weighed and dissolved in 100 ml of double distilled water.

Green Synthesis:-

Preparation of 10 mM $HAuCl_4$ (aq) at room temperature under static Condition.

About 1 gm of $HAuCl_4$, was weighed and dissolved in 100 ml of double distilled water.



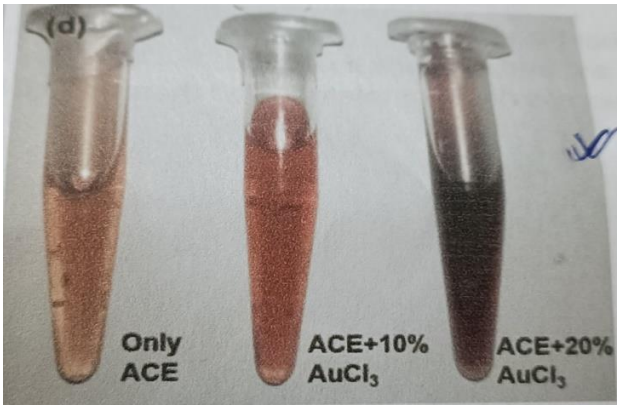


Figure 1

In a conical Flask 20 ml of ACE (plant extract) was added to about 5 ml of aq solution and was put for overnight. Appearance of purple coloured ppt after 7-8 hours.

The nanoparticle emulsion was oven dried at 40ºC for one day. The dried sample was collected & examined for structure and composition for using Powder x-ray diffraction spectroscopy.

The sample was further analysed by uv-visible spectroscopy and FTIR. ACE was analysed for bioreduction Compounds responsible for reaction and also analysed using FTIR spectroscopy.

UV- VISIBLE SPECTROSCOPY:-

The ACE of alovera was mixed with 1 mM gold chloride solution (HAuCl4) and the visible color change was observed due to the formation of nanoparticles. To optimized the concentration of the plant extract, the experiment was carried out by varying the concentration of the extract against a fixed concentration of gold chloride. With the optimised quantity of ACE and 1 mM $HAuCl_4$ aqueous solution, an additional reaction was carried out under continuous stirring condition at 200 rpm (C-MAGHS7, IKA®) at room temperature. The synthesis of the AuNPs was characterized by various spectroscopic (Cary 100 BIO UV–vis, Varian, CA, USA) studies.

SINGLE WAVE LENGTH

CELL NO	WAVELENGTH	ABSORBANCE	(% T)
1-0	540.0	1.693	2.0
1-0	540.9	1.693	2.0
1-0	540.0	1.693	2.0

Table 1

MULTI-WAVELENGTH

CELL NO	WAVELENTH	ABSORBANCE	(% T)
1-0	500	1.702	2.0
1-0	600	1.653	2.2

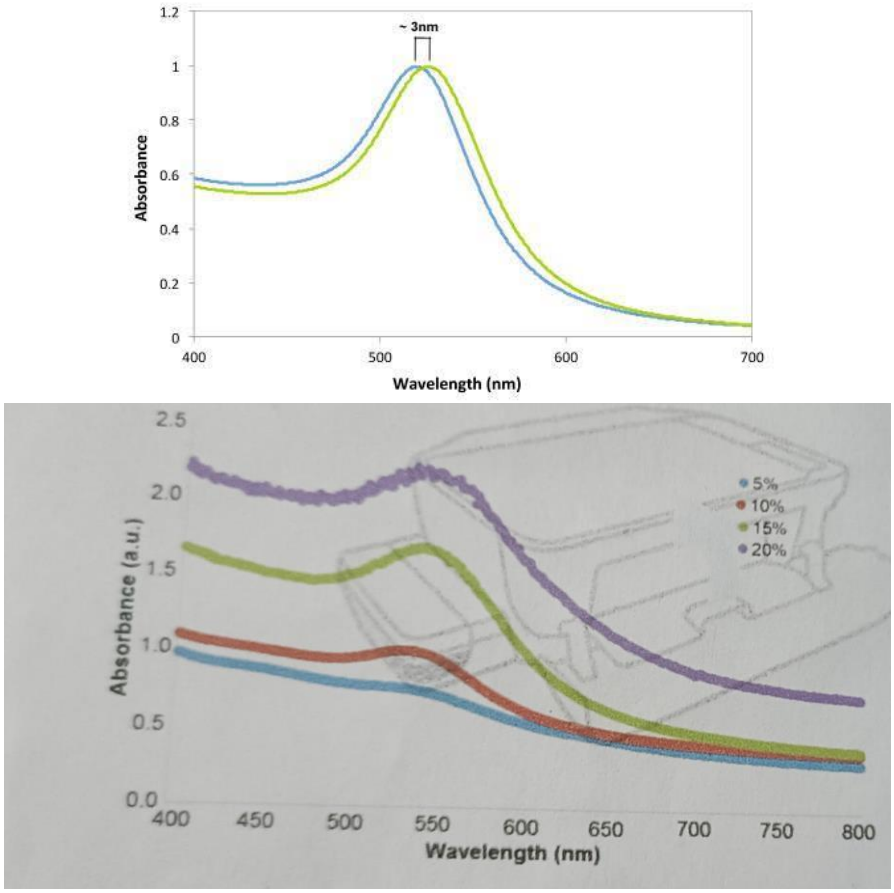


Figure 2 Uv visible spectra of gold nanoparticle

XRD analyses :- The synthesized colloidal AuNPs were centrifuged at 20 000 rpm for 20 min, washed with double-distilled water 2–3 times and freeze dried which were then subjected to XRD analysis. The diffraction of the AuNPs was recorded with a Bruker D8 ADVANCE X-ray powder diffraction using Cu-Kα (λ = 1.54 Å) source in the region of 2θ from 30° to 75°.

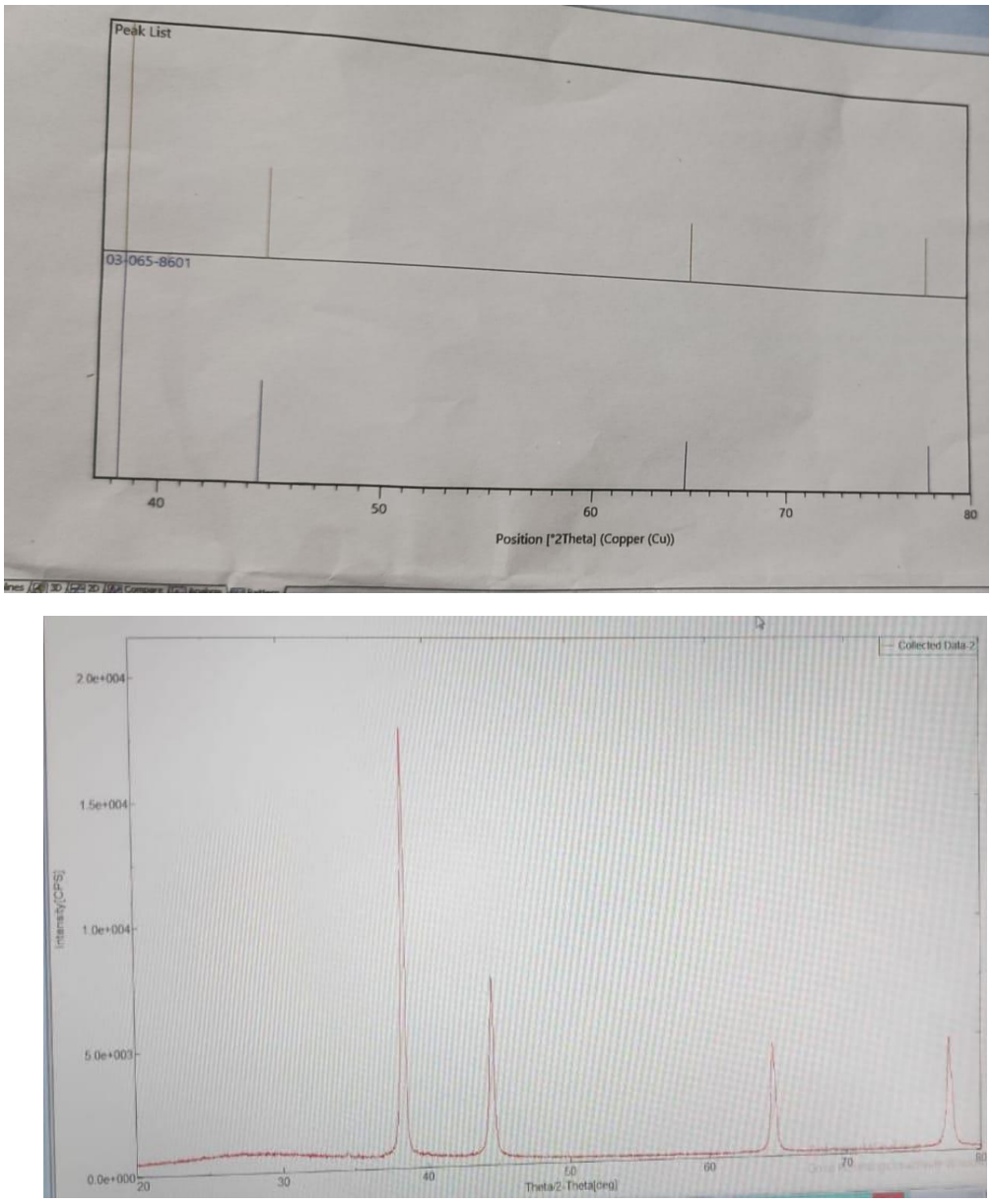
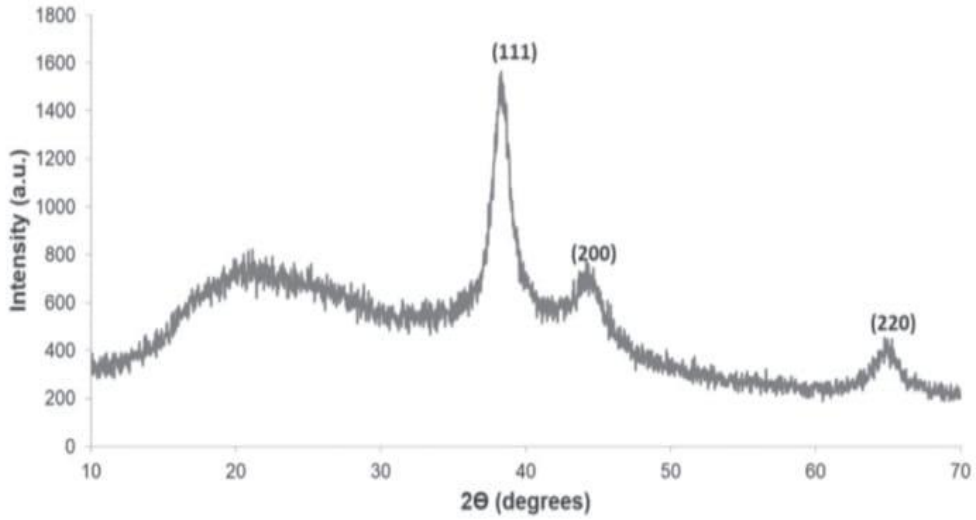


FIGURE 3

Crystalline nanoparticles represented by four peaks corresponding to standard Brag reflections (111), (200), (220), and (311) of face centers cubic lattice. The intense peak at 38.1 represents preferential growth in the (111) direction. The most influential parameters for the synthesis of GNPs were pH 8 , 100º c and 100 ppm aurochlorate salt. The results were veirfied using UV-vis spectroscopy, XRD and FTIR.



XRD pattern of synthesized AuNPs showing peaks of (111), (200), (220) confirming the crystallinity of the particles.

Figure 4

Analysed XRD:-

NAME AND FORMULA

Reference code : 03-065-8601

Compound name: Gold

PDF index name: Gold

Empirical formula: Au

Chemical formula: Au

Crystallographic parameters

Crystal system: cubic

Space group: Fm-3m

Space group number:225

a (Å) : 4.0720

b (Å) : 4.0720

c (Å) : 4.0720

Alpha (º): 90.0000

Beta (º): 90.0000

Gamma (º): 90.0000

Calculated density (g/cm^3) : 19.37

Volume of cell (10^6 pm^3) : 67.52

Z: 4.00

RIR : 25.30

Subfiles and quality

Subfiles: Alloy, metal or intermetallic

Inorganic

NIST Pattern

Quality: Calculated (C)

Comments

Creation Date : 01-01-1970

Modification Date : 01-01-1970

A 7123 53929 15. Temperature Factor : TF TF was not given , B set to 1.000 for calc

Temperature of Data Collection : 20ºC.

References

Primary reference: calculated from NIST using POWD-12++

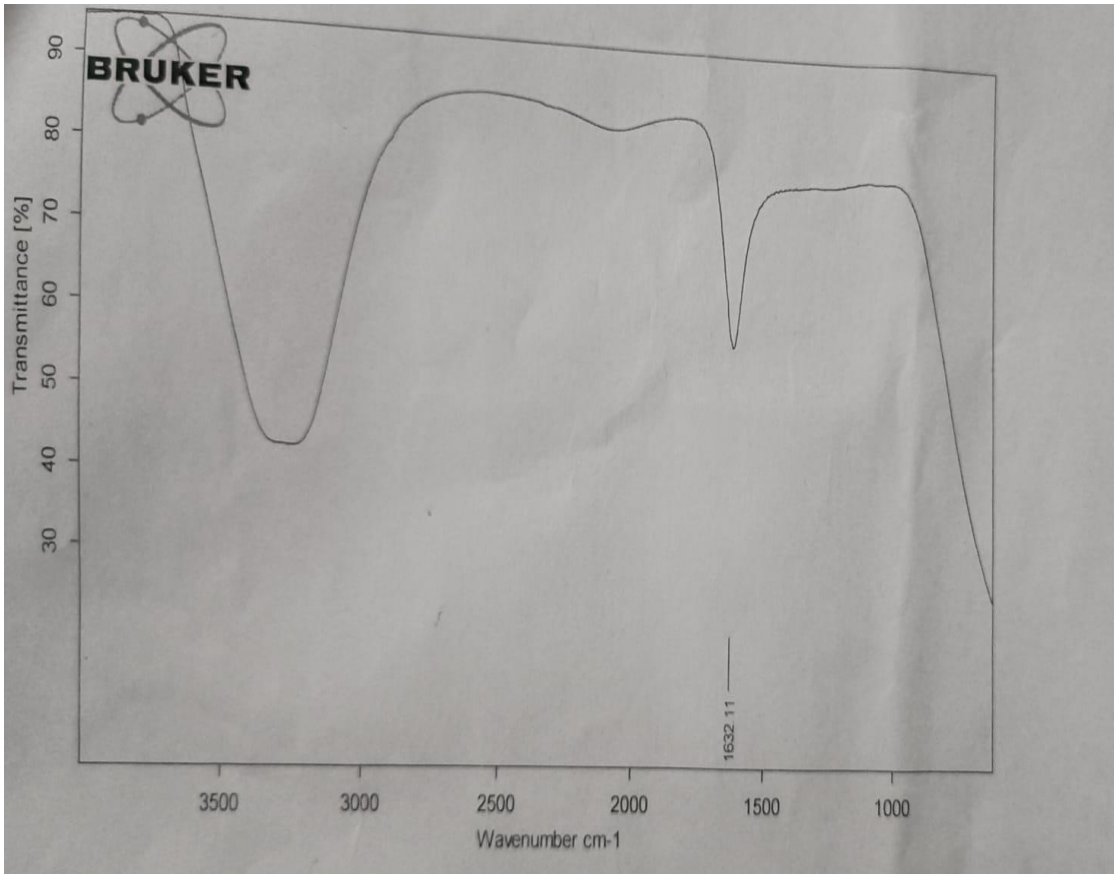
Structure: Suh, I. –K, Ohta, H., Waseda, Y., J. Mater, Sd., 23, 757. (1988)

Peak list

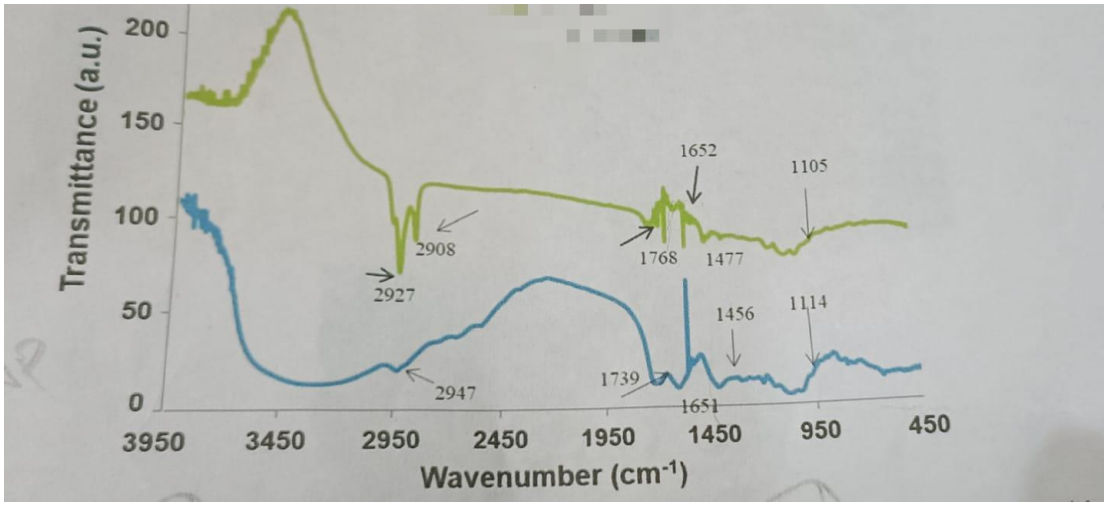
NO.	h	k	1	d [A]	2Theta [deg]	I [%]
1	1	1	1	2.35097	38.253	100.0
2	2	0	0	2.03600	44.462	46.1
3	2	2	0	1.43967	64.695	23.8
4	3	1	1	1.22775	77. 718	24.3
5	2	2	2	1.17549	81.885	6.7
6	4	0	0	1.01800	98.345	2.5
7	3	3	1	0. 93418	111.090	8.8
8	4	2	0	0.91053	115.557	8.3
9	4	2	2	0.83119	135.864	7.1

FTIR:-

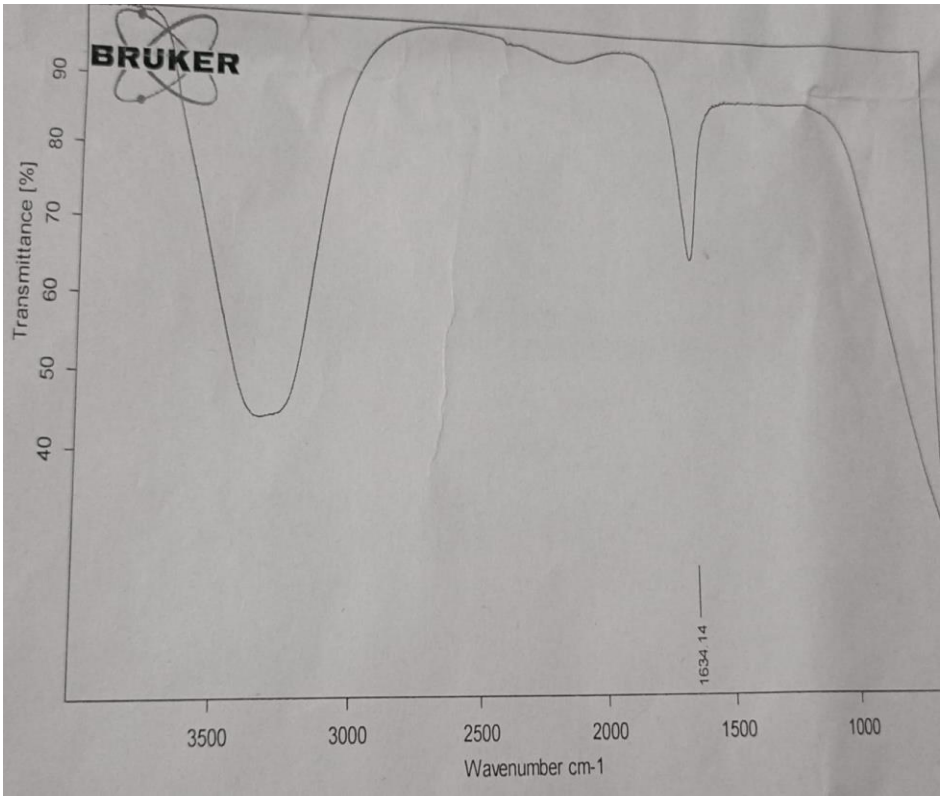
For Fourier transform infrared measurements the AuNP solution was centrifuged at 20 000 rpm for 20 min. The pellet was washed with deionized water, to remove the excess extracts not capped on the surface of the AuNPs. The pellet was re-dispersed in deionized water. The purified sample was lyophilized to obtain GNPs in powder form.



FTIR spectra of plant extract



FTIR specrtra of synthesized AuNPs and only plant extract confirming the capping of phenolic compounds on the nanoparticles.



FTIR spectra of gold nanoparticle

Figure 5

RESULT AND DISCUSSION:-

Reduction property of Aloe barbadensis miller (alovera) ACE:-

The reduction of $AuCl_4$, was visually evident from the color changes and the stable ruby red color indicated the formation of AuNPs Synthesis of the AuNPs was confirmed by scanning the absorption maxima of the reacted mixture at the wavelength between 200-800 mm on a Cary 100 BIO UV Vis spectrophotometer (Varian, CA, USA). Spectroscopic scanning of the colored solution exhibited distinct surface plasmon resonance (SPR) bands with an absorption peak centered at around 530-535 nm, characteristic of spherical AuNPs (figure 2). At the plant extract concentration above 20%, the increase in SPR intensity was negligible, indicating an attainment of saturation in the bio-reduction of $AuCl_4^-$

Crystallinity analysis of AuNPs by XRD. XRD patterns of the AuNPs synthesized with aloe barbadensis ACE concentration of 20% displayed Bragg reflections representative of the fcc structure of gold (figure 4). The intensity of the peak of (111) at 38.3° diffraction was much stronger than those peaks of (200) and (220) at 44.3° and 65.4°, respectively. In the case of the optimum aqueous core extract, the average diameter obtained from XRD analysis was in the range of 5-50 nm, respectively. As the Debye-Scherrer equation is best applicable to highly monodispersed nanoparticles, in the present study the average diameter of polydispersed AuNPs may be physically more relevant. Such deviation can be attributed to highly polydispersed AuNPs within adequate adaptation to a particular geometry [13, 18]

FTIR spectra analysis of synthesized AuNPs:-

The FTIR spectra (figure 5) of the plant extract of aloe barbadensis showed characteristic bands for C-H alkanes, C=O carbonyl. N-H primary amines, N-O nitro group, C-C aromatic and C-O stretch for alcohols, carboxylic acids, esters and ethers at 2927 and 2908, 1768, 1652, 1558, 1477, and 1105 cm^{-1} respectively [19, 20]. The corresponding peaks for the said spectra appeared due to the presence of various phytochemicals present in the crude extract. The FTIR analysis of the AuNPs revealed the presence of all bands common to the aqueous plant extract of aloe barbadensis C-H of) alkane at 2947 cm^{-1}. C-O carbonyl at 1739 cm^{-1}, 1651 cm^{-1} for N-H primary amines, 1456 cm^{-1} for C-C aromatic and 1114 cm^{-1} for alcohol, carboxylic acid, esters and ethers suggesting the presence of phytochemicals on AuNPs surface. The capping behavior and stability of the synthesized AuNPS could be due to the presence of these phytochemicals in the crude extract.

Pharmacological studies of AuNPs

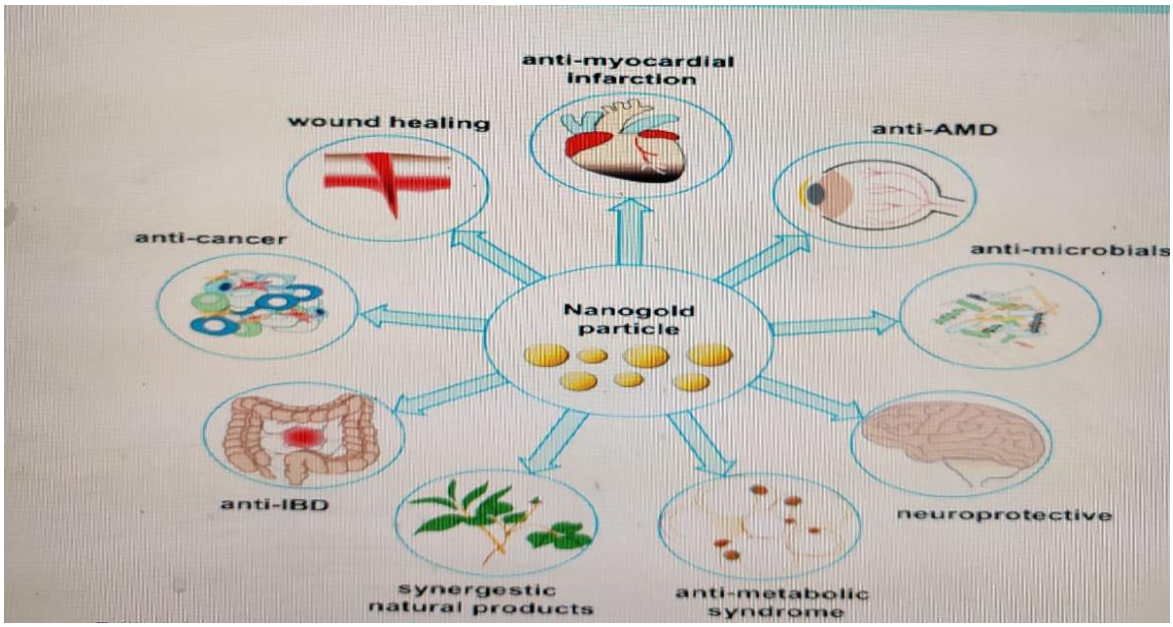


table 4 :- summary of the effects and mechanism of gold nanoparticles in diseases

Diseases	Applications (Future) or Possible Action Mechanisms
Cancers pancreatic, breast, prostate, colon, melanoma, sarcoma, and lung cancers, etc.	Anti-cancer activity; cancer diagnosis, imaging applications, photothermal and photodynamic therapies; anti-cancer drug and gene delivery
Retinopathy age-related macular degeneration (AMD); diabetic retinopathy (DR)	Anti-angiogenesis; anti-inflammation; reduced the VEGF activation and induced cell proliferation and migration
Neurological diseases Alzheimer's disease; Parkinson's disease	Inhibited the aggregation of Aβ peptides and the degradation of Aβ aggregates; inhibition of acetylcholinesterase and butyrylcholinesterase; anti-inflammation
Skin disorders	Wound healing; acne; synergistic effect with natural products
Bowel diseases	Against inflammatory bowel diseases (IBD); alleviates the lipopolysaccharide-induced intestinal epithelial barrier dysfunction
Bone cartilage disorders	Rheumatoid arthritis treatment; Promotion and regulation of the differentiation; protection for bone and cartilage tissue; the inhibition of osteoclast; inhibit angiogenic activities, suppress inflammation or serve as antioxidant
Cardiovascular diseases	CT imaging as CT contrast agents; anti-inflammatory biological activity; reduce arterial neointimal hyperplasia
Infections	Antimicrobial effects; overcome microbial drug resistance; detect specific DNA fragments of *Mycobacterium tuberculosis*; antiviral activity; coronavirus vaccines and the detection
Metabolic syndrome	Type 2 diabetes and obesity treatment; improvement in glucose intolerance and hyperlipidemia; lipolysis; more effects during liposuction

CONCLUSION:-

Nanobioscience has drawn increasing attention due to its avant-garde nature and the efficacy of nano particles in industrial, biomedicials and eletronic applications such as a Catalyst (21), for Cancer detection(22, 23) and as bioiamaging and theranostic agents. As an alternative to Chemical reductants, green phytochemical reducing agents have gained much interest in the synthesis of nanoparticles due to their capability to promote sustainability initiatives. Green reductants consist of various biological entities ranging from bacteria and fungi to plant parts (fruits, leaves, petals), algae etc (6,24). The high phenolic Content of the aqueous core extract of Alo vera having strong anti oxidant property helped in the reduction of gold cations to AuNPs. This green and time saving method for AuNPs synthesis does not require any toxic or hazardous reducing Chemical agent and thus has potential for use in biomedical applications. The various phyto chemicals present in Aloe barbadensis leaves extract mediate the surface Capping of AuNPs which was evident from FTIR studies (25). The crystalline nature of the AuNPs was Confirmed from the XRD pattern.

This eco friendly green small nanoparticles will reach various organs throughout the body, including the brain,heart, lungs, spleen, and kidneys, where the smaller particles are further eliminated through the kidneys. It will further reach the location of tissue disease [59]. Topical applications of nanoparticles such as ophthalmic formulations can directly achieve the therapeutic goals discussed in this paper due to their direct contact with the target site. The toxicity of gold nanoparticles may be closely related to factors such particle size, shape, surface potential, dose, and synthesis method. Therefore, the clinical application of gold nanoparticles requires more detailed toxicological tests to prove its safely. At present most of the related preparations of gold nanoparticles, are still in the clinical trial stage. In the future, more nano-related preparations will enter the clinical treatment and diagnosis of diseases, so that nanogold will play a more prominent role in biomedicine.

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