ANTIMICROBIAL STUDIES OF ZrO_2 NANOPARTICLES AND THEIR PHYTOCHEMICAL STUDIES

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ABSTRACT

Since it looks like the shade of the tooth, it has a persuading machine in thickness. However it uncovered such differentiated applications, just restricted works have been performed on Zirconium dioxide nanoparticles to check for their antibacterial and in vitro cell reinforcement movement. In any case, there is proof that Zirconium Zr (IV) projects strong antibacterial as well as an antifungal action when blended in with ligand buildings [1-4]

For the most part, nano zirconia is created by different substance, for example, solvothermal aqueous [5-9], watery precipitation [10] sol-gel [11-13], and warm deterioration strategies [14-16] . However, these techniques enjoy different benefits, for example, savvy, mass creation and conveyance of profoundly controlled size and state of nanoparticles [17], they frequently discharge risky synthetic squanders which are hurtful to the climate. To devalue these issues, the job of greener methodologies in nanoparticle manufacture becomes unavoidable. Among the different bio - based strategies, fabricating nanoparticles by utilizing plant removes is the most fit strategy for enormous - scale creation because of the simple accessibility of plants, non - prerequisite of aseptic circumstances, and simple dealing with [18].

Moringa oliefera generally known as drumstick tree has a place with the group of Asclepiadaceae. Since old times every single piece of this plant is viewed as utilized in different clinical applications. It is now revealed that the blossoms, seeds and leaves were utilized in the treatment of stomach cancers, restoring cerebral pains and furthermore went about as compelling for purgative [19]. Phyto compound investigations of the watery leaf concentrate of Moringa oliefera portrayed upgraded anti-ulcerogenic impact [20] in this manner remains as a proof for its utilization in conventional medication. Supposedly, without a doubt, not many works have been enlisted on the Phyto helped combination of ZrO_2NPS and this was one of those deals with ZrO_2 nano particles creation utilizing Moringa oliefera leaf remove.

3.1. Andrographis paniculate:

An herb is a plant or plant part used for its scent, flavor, or therapeutic properties, and medicinal products made from them are frequently used to improve health as dietary supplements. Andrographis paniculata (Burm. f) Nees., also known as Kalmegh or the "King of Bitters," is a herbaceous plant in the Acanthaceae

family [21]. Mostly leaves and roots have been used for centuries for various medicinal purposes in Asia and Europe as a folklore remedy for a wide range of ailments or as a herbal supplement for health promotion [22].

3.2. Synonyms

English: The King of Bitters; Gujrati: Kariyatu; Hindi: Kiryat; Japan: Senshinren; Kannada: Nelaberu; Malayam: Kiriyattu; Malaysia: Hempedubumi, Sambiloto; Marathi: Oli-kiryata; Oriya: Bhuinimba; Persian: Naine-havandi; Sanskrit: Kalmegha, Bhuinimba; Scandinavian: Green [23]

3.4. Taxonomical Classification

Kingdom: Plantae, Plants;

Sub Kingdom: Tracheobionta, Vascular plants;

Super Division: Spermatophyta, Seed plants;

Division: Angiospermae

Class: Dicotyledonae

Subclass: Gamopetalae

Series: Bicarpellatae

Order: Personales

Tribe: Justicieae

Family: Acanthaceae

Geinus: *Andrographis*

Species: *A. paniculata* (Burm.f) Nees

3.5. Habitat

It grows abundantly in southeastern Asia, including India, Sri Lanka, Pakistan, Java, Malaysia, and Indonesia, but it is also widely cultivated in India, China, Thailand, the East and West Indies, and Mauritius. Andrographis paniculate is normally grown from seeds in native areas where it grows in pine, evergreen, and deciduous forest areas, roads, and villages. It is grown in India during the rainy season of the summer (Kharif crop). This crop can be grown commercially in any soil that contains a reasonable amount of organic matter.

3.6. Morphology

It is an annual herbaceous plant that grows upright to a height of 35 to 100 cm in wet, shaded areas. Its stem is abruptly quadrangular, heavily branched, and delicate in texture. Simple, opposite, lanceolate, glabrous leaves are 2-10 cm long, 1.5-2.5 cm wide, with a sharp, whole, or hardly undulating border. Upper leaves are frequently bractiform and have a short petiole. The plant's inflorescence is described as plain, terminal, and axillary in panicle, measuring 10–30 mm long; the bract is tiny; and the pedicel is short. The flowers have the following botanical characteristics: a 5-part, short, linear calyx; a narrow, 5-mm-long corolla tube; a limb longer than the tube; bilabiate lips on the upper and lower surfaces; stamens 2 placed in the throat and far exserted; and an anther basally beared. The plant's capsule is upright, linear-oblong, compressed, longitudinally grooved on broadsides, sharp at both ends, and thickly glandular-haired. It is

1.0-3 cm long and 3–4.5 mm wide. The seeds of Vietnamese medicinal herbs are quite tiny and subquadrate. [24-26].

(a) **(b)**

(c) (d)

Figure: 3.1. Morphology of *Andrographis paniculata* showing a**)** Arial parts with mature and immature capsules; b**)** flower buds to flowering to capsule formation; c**)** matured seeds; d**)** leaves.

3.7. Demand

A paniculata is sought for its therapeutic qualities. It has long been used in Asia to treat a variety of chronic and irresistible diseases. including fever, herpes, sore throats, upper respiratory infections, and gastrointestinal tract infections. According to the Indian Pharmacopoeia, it is the main ingredient in at least 26 Ayurvedic medicines.[27] It is a crucial herb with "cool properties" in traditional Chinese medicine that is used to remove toxins from the body and reduce body heat, such as in fevers. It is frequently utilized to both control and treat the ague in Scandinavian nations [28]. The MPH in Thailand decided to add this plant as one of the therapeutic flora on "The National List of Essential Drugs A.D. 1999".[29,30]

3.8. Commercial importance and market potential

The market quiescent of *A. paniculata* is very high it is highly consumed as stomachic, hepatoprotective, dyspepsia, anthelmintic, bitter tonic, and febrifuge. With reference to trade, an estimated consumption of *Andrographis paniculate* aerial parts is 250 tones.[31-33] The major, biologically active plant metabolites

isolated from poles parts of this plant are andrographolide, 14-deoxy-11-oxoandrographolide,14-deoxy-11,12didehydroandrographolide,and neoandrographolide[34].

The other important compounds isolated from different parts of *A. paniculate* are apigenin-7, 40-di-methyl ether, carvacrol, eugenol, myristic acid, hentriacontane, tritriacontane, oroxylon A, and wogonin [35]. The extraction of andrographolide from wild populations routinely satisfies the pharmaceutical industries' strong demand for the chemical; nonetheless, the limited commercial viability of this substance is the apprehension of its scarcity. Indian farmers have begun commercial fabrication of this medicinal plant due to the high demand for andrographolide in both home-style and foreign markets.

3.9. Pharmacological ascribe

A surprising assortment of pharmacological effects, some of which are quite beneficial, was said to be generated by Andrographis when used properly, according to research done in previous decades:-

Abortifacient: can carry out the pregnancy.

Acrid: In this case, obscurely rubefacient to the skin.

Analgesic: Analgesic.

Antibacterial: Fights bacterial activity.

Antidiarrhoeal and intestinal effects: Useful in avoiding diarrhea and bacterial dysentery.

Anti-inflammatory activity: Andrographis paniculata is also used as a folk medicine for fever, pain reduction, and disorders of the intestinal tract.

Antimalarial activity: Andrographis paniculata is found to considerably inhibit the multiplication of Plasmodium berghei.

Antioxidant activity: Battle the free radicals.

Antipyretic: Fever abridge.

Antisnakevenom: Kill the snake venom.

Antithrombotic: Blood clot circumvention.

Antiviral: Control viral activity.

Cancerolytic: Fights and destroy the cancer cells.

Cardiovascular activity: Efficacious in lowering cholesterol.

Choleretic: Change the properties and flow rate of bile.

Depurative: The system is purged and kept fresh, mainly the blood.

Expectorant: Stimulates the outflow of mucus from the nasal passages.

Hypoglycemic activity: Avoiding hypoglycemia.

Hepatoprotective exercise: In Ayurvedic medicine, there are 26 different remedies containing Andrographis paniculata used to treat liver disorders.

Immunological potential: increases white cell phagocytosis, inhibits HIV-I replication, and Enhance CD4+ and T-lymphocytes counts.

Laxative: helps with colon discharge.

Psycho-pharmacological activity: Potent central nervous system depressant action.

Sedative: Although it doesn't have the same impact as commonly used herbal sedatives like valerian root, hops, skullcap, etc., skullcap is a calming plant.

Thrombolytic: Blood clot buster.

***Vermicidal*:** Kills intestinal worms.

3.10. Phytochemical attributes

The trademark auxiliary metabolites experienced in the plant have extensively improved its significance in the field of restorative plants and prescriptions. It is explicitly appraised exceptionally high in helpful activity in restoring liver problems and normal hack and cold in people. Various diterpenoids and diterpenoid glycosides of comparable carbon skeleton have been disconnected Andrographis, primarily the harshest mixtures among them are andrographolide, neoandrographolide, deoxyandrographolide. The elevated pieces of the plant are utilized to separate the dynamic phytochemicals. Previous investigations on the chemical composition of *Andrographis paniculata* showed that it is a rich source of diterpenoids and 2'-oxygenated flavonoids including andrographolide, neoandrographolide, 14-deoxy-11,12-didehydroandrographolide, 14-deoxyandrographolide, is andrographolide and 14-deoxyandrographolide 19 β- D-glucoside, homoandrograholide, andrographan, andrographosterin, stigmasterol [36] andrographiside, deoxyandrographiside, homoandrographolide, andrographan, andrographon, andro-graphosterin. The bioactive compound of the medicinal plant *Andrographis paniculata* is andrographolide. Andrographolide has a lactone function, is faded and crystalline in appearance, and has a very bitter taste. The leaves of Andrographis contain the most elevated measure of andrographolide, the most restoratively dynamic phytochemical in the plant, while the seeds contain the least. The molecular formula of andrographolide is $C_{20}H_{30}O_5$, while its molecular structure is shown in Fig: 3.2. Andrographolide can be easily dissolved in methanol, ethanol, pyridine, acetic acid, and acetone, but slightly dissolved in ether and water.

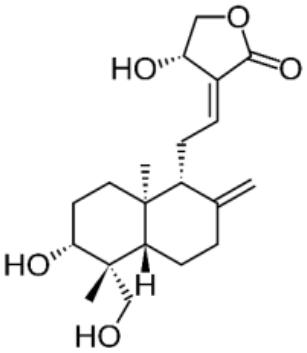

Figure:3.2 Andrographolide compound

Figure:3.3. *Andrographis paniculate leaf powder*

ANTIBACTERIAL ACTIVITY

4.1. MATERIALS AND INSTRUMENTS:

Nutrient agar media, Nutrient broth, DMSO, well-borer, Petri plates, conical flasks, pipettes, Eppendorf tubes, glass bottles, BOD incubator, and laminar air flow, autoclave, and hot air oven.

4.2. PROCEDURE:

Antibacterial activity of test compounds on bacterial strains(Escherichia coli and Staphylococcus aureus) was carried out by the cup plate agar diffusion method. Nutrient agar medium prepared was inoculated with 18hrsold cultures of the test organism and poured into a Petri plate. The medium in the plates was allowed to solidify at room temperature. After the media gets solidified 4 cups of 5mm diameter were made on each plate at an equal distance. The stock solutions of the test compound were prepared in concentrations of 800 µL, 600 µL and 300 µL. Therefore 50 µL of each concentration were placed in the cups by means of sterile micropipettes. On each plate, one cup was used for standard drug. The Petri-dishes thus prepared were incubated in an incubator for 24 hrs at 37℃ and later examined by measuring the zones of inhibition.

4.3. RESULT:

The area around the antibiotic disk that has no bacterial growth is known as the zone of inhibition. The larger the zone is, the more sensitive the bacterium is to that test compound. The smaller the zone is, the more resistant (and, thus, less sensitive) the bacteria. It was clear from Table 2, Figure 5 a) and b), that the inhibition zones for the gram-negative bacteria (Escherichia coli) were bigger and showed more activity than that of the gram-positive bacteria (Staphylococcus aureus).

S.No	TestCompound	Organism	800µL	600µL	300µL	CONTROL
1	RC-8	Escherichiacoli	1.2mm	1.0mm	0.9mm	2.5mm
2	RC-8	Staphylococcusaureus	-	-	-	3.0mm

Table.2: Antibacterial activity showing the diameter of the zones on an organism of bacteria i.e., gram-negative (E.coli) and gram-positive(S.aureus).

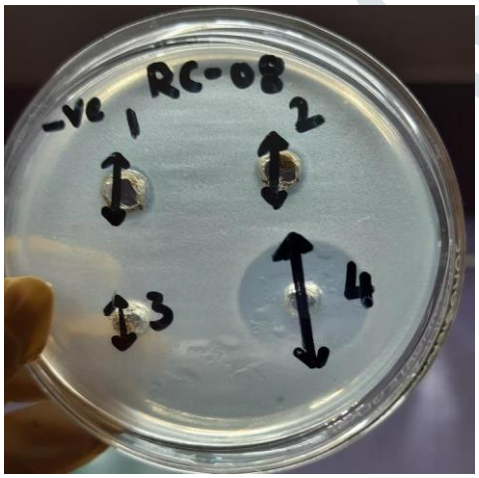

Fig.5 a). Test compound RC-08 againstEscherichia coli.

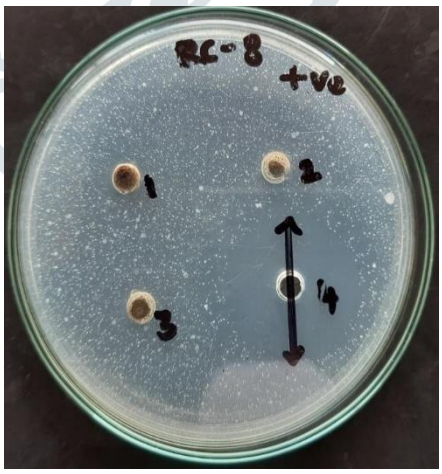

Fig.5 b). Test compound RC-8 against Staphylococcus aureus.

5. ANTIFUNGAL ACTIVITY

5.1. MATERIALS AND INSTRUMENTS:

Potato dextrose agar, potato dextrose broth, DMSO, Distilled water, Well-borer, conical flasks, pipettes, Eppendorf tubes, BOD incubator, Laminar air flow, autoclave, and hot air oven.

5.2. PROCEDURE:

The potato dextrose agar medium was prepared and inoculated with 50 µL of the fungal test organism Aspergillus niger and Rhizoctonia solani, which were prepared from 48 hours-old cultures were thus transferred into sterile Petri plates. The medium is allowed to solidify at room temperature. The 4 cups of 5mm diameter were made on each plate at equal distances. Stock solutions of test residual extract were prepared in concentrations of 800 µL, 600 µL, and 300 µL of each of the above. Stock concentrations were placed in the cups by means of sterile pipettes. On each plate, one cup is left for control. Therefore, the plates were incubated for 24 to 48 hours at 35 ℃. The experiments were run in duplicate and the average diameter of the zones of inhibition were recorded in tabular form.

5.3. RESULT:

The area around the disk that has no fungal growth is known as the zone of inhibition. The larger the zone is, the more sensitive the organism is to that test compound. The smaller the zone is, the more resistant (and, thus, less sensitive) the organism is. . It was clear from Table 3, Figure 6a) and b) that the inhibition zones for the Aspergillus niger have shown more activity among the test compounds than that of the organism Rhizoctonia solani.

S.No	Test Compound	Organism	800 µL	600 µL	300 µL	CONTROL
1.	RC-8	Aspergillus niger	2.0 mm	1.6 mm	1.0 mm	3.0 mm
2.	RC-8	Rhizoctonia solani	1.5 mm	1.1 mm	0.9 mm	3.0 mm

Table.3: Antifungal activity showing the diameter of the zones on organisms of fungi i.e. Aspergillus niger and Rhizoctonia solani.

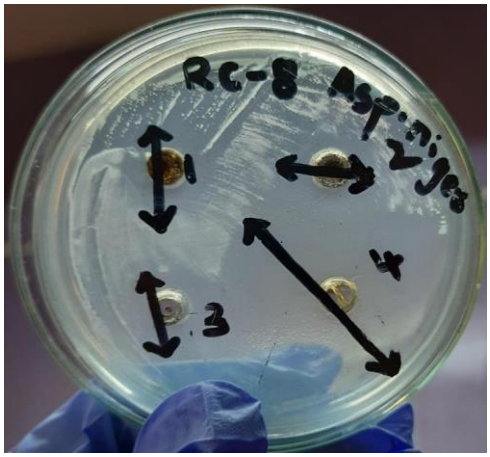

Figure: 6. a). Test compound RC-08 against Aspergillus niger.

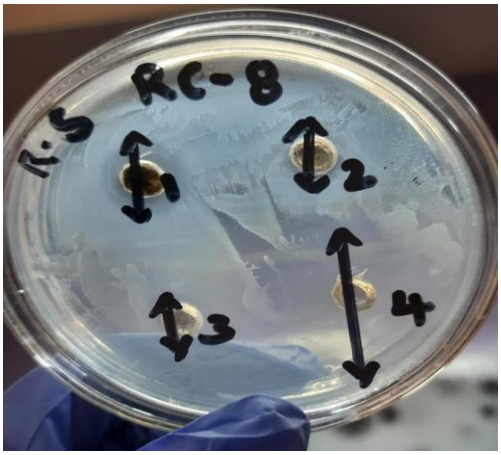

Figure: 6.b) Test compound RC-08 against Rhizoctonia solani.

References:

1. Z.Q. Ye, M.Q. Tan, G.L. Wang, J.L. Yuan, J. Fluoresc. 15 (2005) 499–505.
2. F. Ramos-Brito, C. Alejo-Armenta, M. García-Hipólito, E. Camarillo, J. Hernández,S. Murrieta, C. Falcony, Opt. Mater. 30 (2008) 1840–1847.
3. T. Lopez, M. Alvarez, F. Tzompantzi, M. Picquart, J. Sol–Gel Sci. Technol. 37(2006) 207–211.
4. H. Zhang, Z. An, F. Li, Q. Tang, K. Lu, W. Li, J. Alloy. Compd. 464 (2008) 569–574.
5. L. Lerot, F. Legrand, P. Debruycker, J. Mater. Sci. 26 (1991) 2353–2358.
6. O. VanCantfort, B. Michaux, R. Pirard, J.P. Pirard, A.J. Lecloux, J. Sol–Gel Sci.Technol. 8 (1997) 207–211.
7. B.W. Yan, C.V. McNeff, P.W. Carr, A.V. McCormick, J. Am. Ceram. Soc. 88 (2005)707–713.
8. J. Widoniak, S. Eiden-Assmann, G. Maret, Eur. J. Inorg. Chem. (2005) 3149–3155.
9. C. Zhang, C. Li, J. Yang, Z. Cheng, Z. Hou, Y. Fan, J. Lin, Langmuir 25 (2009)7078–7083.
10. A. Parmaa, I. Freris, P. Riello, F. Enrichi, D. Cristofori, A. Benedetti, J. Lumin. 130(2010) 2429–2436.
11. S.F. Wang, F. Gu, M.K. Lu, W.G. Zou, S.W. Liu, G.J. Zhou, D. Xu, D.R. Yuan, Opt.Mater. 27 (2004) 269–272.
12. Z.W. Quan, L.S. Wang, J. Lin, Mater. Res. Bull. 40 (2005) 810–820.
13. T.H. Guo, Y. Liu, Y.C. Zhang, M. Zhang, Mater. Lett. 65 (2011) 639–641.
14. Y.C. Zhang, X. Wu, X.Y. Hu, R. Guo, J. Cryst. Growth 280 (2005) 250–254.
15. Y. Ang, G.P. Feng, T.X. Wang, Mater. Lett. 64 (2010) 1647–1649.
16. H. Bai, X. Liu, Mater. Lett. 64 (2010) 341–343.
17. S.S. Devi, S.B. Paul, Biol. Environ. Sci. 7 (I) (2011) 156–160.
18. J. Sun, X. Gao, D. He, J. Chen, X. Meng, Q. Zhang, L. Shen, H. Jiao, Particuo 11(2013) 776–781.
19. N. Salah, S.S. Habiba, Z.H. Khana, F. Djouider, Radiat. Phys. Chem. 80 (2011)923–928.

20. I. John Berlin, L.V. Maneeshya, J.K. Thomas, K. Thomas, K. Joy, J. Lumin 132 (2012)3077–3081.
21. Mishra, S.K., Sangwan, N.S. and Sangwan, R.S. 2007. *Andrographis paniculata* (Kalmegh): *A review*. *Pharmacog. Rev.*, 1: 283-289.
22. Kanokwan, J. and Nobuo, N. 2008. Pharmacological aspects of *Andrographis paniculata* on health and its major diterpenoid constitute andrographolide. *J of Health Sci.*, 54 (4): 370-381.
23. Traditional Thai Medicine: Fah Tha Lai and Nam Rai Pangpond (Kanokwan and Nobuo, 2008).
24. Pharmacopoeia of the People's Republic of China. 1997. Vol.1 (ed). Beijing, Chemical Industry Press.
25. Standard of ASEAN herbal medicine 1993. Vol. 1. Jakarta, ASEAN Countries.
26. Thai herbal pharmacopoeia 1995. Bangkok, Prachachon Co. Vol. 1.
27. Wongkittipong, R., Prat, L., Damronglerd, S. and Gourdon, C. 2000. Solid – liquid extraction of andrographolide from plants experimental study, kinetic reaction, and model. *Separation and Purification Technology.*, 40: 147-154.
28. Faisal, M., Ahmad, N. and Anis, M. 2005. Shoot multiplication in *Rauvolfia tetraphylla* L. using thidiazuron. *Plant Cell Tiss. Org. Cult.*, 80: 187-190.
29. Kataky, A. and Handique, P.J. 2010a. Micropropagation and screening of antioxidant potential of *Andrographis paniculata* (Burm. f) Nees. *Journal of Hill Agriculture.*, 1(1): 15-20.
30. Kataky, A. and Handique, P.J. 2010b. Antimicrobial activity and phytochemical estimation of micropropagated *Andrographis paniculata* (Burm. f) Nees. *Asian Journal of Science and Technology.*, 5: 91-94.
31. Sharma, A., Shanker, C., Tyagi L. K., Singh, M. and Rao, Ch. V. 2008. Herbal Medicine for Market Potential in India: An Overview. *Academic Journal of Plant Sciences.*, 1 (2): 26-36.
32. Agrawal, S. S., Tamrakar B. P. and Paridhavi, M. 2005. Clinically Useful Herbal Drugs. Ahuja Publishing house, Delhi, 1st Edn.
33. Kandya, A. K. 2005. Cultivation of Some Medicinal Plant Species and Requirement of Seeds. *Pharmacognosy Magazine.*, 1(2): 38-44.
34. Balmain, A and Connolloy, J.D. 1973. Minor diterpenoid constituents of *Andrographis paniculata* Nees. *J. Chem. Soc. Perk. Tr. I.* 12: 1247-1251.
35. Rastogi, R.P. and Mehrotra, B.N. 1993. Compendium of Indian medicinal plants, New Delhi: CDRI and Publication and Information Directorate. 3: 1980– 1984.
36. Pholphana, N., Rangkadilok, N., Thongnest, S., Ruchirawat, S., Ruchirawat, M. and Satayavivad, J. 2004. Determination and variation of three active diterpenoids in *Andrographis paniculata* (Burm. f) Nees. *Phytochem. Anal.*, 15: 365-371.