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PHYTOCHEMICAL AND ANTIMICROBIAL PROFILE OF NANOBASED LIV-PRO-08 POLYHERBAL FORMULATION

Suriyavathana Muthukrishnan[1], Anandhi Eswaran[2]

[1] Department of Biochemistry, Periyar University,
Salem, Tamil Nadu, India.
[2]Assistant Professor,Department of Biochemistry,
Periyar University,Salem – 11, Tamilnadu,(India)

ABSTRACT

The phytochemical analysis and Anti microbial activity was performed on green synthesized silver particles of Liv-Pro-08 polyherbal formulation. Silver nanoparticles have shown the efficient presence of phytochemicals (alkaloids, flavonoids, tannins, phenol, cardiac glycosides, terpenoids, carbohydrate, protein). Anti microbial activity was screened with some bacterial (Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumonie) and few fungal strains(Fusarium oxysporum and Aspergillus niger) in silver nanoparticles. The maximum inhibitory zone of was noted to be in Klebsiella pneumonia and Aspergilus niger, which shows AgNPs Liv-Pro-08 possess potent antimicrobial effect. The Phytochemical analysis and antimicrobial activity of green synthesized Liv-Pro-08 polyherbal formulation may have its therapeutic potential against infectious diseases.

Keywords: Anti microbial activity, Entada pursaetha , Ficus glomerata, Nigella sativa, Phytochemical Screening.

I. INTRODUCTION

Ayurveda is a traditional Indian medicinal system which is practiced for thousands of years. In this system of medicine, more than 1200 plants, nearly 100 minerals and over 100 animal products are used. Due to considerable research on pharmacognosy, chemistry, pharmacology and clinical therapeutics, numerous drugs have entered into international pharmacopoeia. Ayurvedic treatment has been estimated to meet 70-80% of the healthcare needs of India [1,2]

According to the World Health Organization **(WHO)**, about three quarters of the world's population currently use herbs and other forms of traditional medicines to treat diseases. Traditional medicines are widely used in India. Even in USA, use of plants and phytomedicines has increased dramatically in the last two decades. It has been also reported **[**3**]** that more than 50% of all modern drugs in clinical use are of natural products.

Infectious diseases are the leading cause of death across the world. As a global concern the antibiotic resistance by pathogens has emerged. Many of the antibiotics have been out of use as multidrug resistant pathogens have emerged. Natural products, either as pure compounds or as standardized extracts, provide unlimited

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opportunities for new drug leads because of the unmatched availability of chemical diversity. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and reemerging infectious diseases. Therefore, much attention has been given to traditional medicine in order to look for new leads to develop better drugs to treat resistant bacteria[4]

Nanotechnology is one of the fastest developing sciences over the last few years. This is an inter-disciplinary science that connects knowledge of biology, chemistry, physics, engineering and material science[5]**.** Nanotechnology deals with the design, production and application of nanoparticles – particles with a size below 100 nm. The reason for this interest is that the physical and chemical properties of particles in the nanorange can be different from larger particles or dissolved compounds[6].

Over the past few years, the metal nanoparticles are focused mainly for the research work due to their potential applications in different fields such as magnetic recording media or micro electronics, catalysis, nanosensors, nanoelectronics, optoelectronics and information storage devices. Some properties such as morphology, size and distribution of the particles are clearly obtained from the nanoparticles. Many researchers used green synthesis methods for different metal nanoparticles due to their growing need of eco-friendly properties. Green synthesis method was found to be the best method when compared to the other method such as chemical reduction, photochemical reduction, electrochemical reduction, heat evaporation etc., In this method, the plant extract has been used as capping and reducing agent for the synthesis of copper and silver nanoparticles due to their reducing properties present in the plant sample extract.

Nigella sativa (N. sativa) L. (Ranunculaceae) is an annual flowering plant, which is native to South and Southwest Asia and is cultivated and used in different parts of the world, such as the Mediterranean countries, southern Europe, and North Africa. Traditionally, it is used as a natural remedy for a number of illnesses that include diarrhea, indigestion, dyspepsia, sour belching, obesity and dyspnoea. In addition, it has been reported that the seeds with bee honey have protective effects on hepatotoxicity and on the oxidative stress and carcinogenesis[7].

Entada pursaetha (Mimosaceae) is a woody climber of the legume family. It is found growing naturally throughout tropical Africa and South-Eastern Asia. *Entada* species have two amorphous saponins and the seeds have various medicinal uses including topical applications in an ointment for the treatment of jaundice. The chemical constituents and biological activity of this plant have not been previously investigated but phytochemical studies on related species revealed the presence of saponins [8,9,10]. Since some triterpene saponins from the family Leguminosae have been reported to be cytotoxic for tumor cell lines[11].

Ficus glomerata Roxb. syn. *F. racemosa* L. (Family: Moraceae), commonly known as Gular in Hindi and Cluster fig in English. Several members of the genus *Ficus* (Ficus glomerata) are being used traditionally in a wide variety of ethnomedical remedies all over the world [12,13] Also, some studies reported the presence of antioxidant activity of some Ficus species which attributed the antioxidant activity to the phenolic content of them [14,15,16]. In the Indian system of medicine, the seeds are used as astringent, bitter, stimulant, diuretic, emmenagogue, anthelmintic, jaundice, intermittent fever, dyspepsia, paralysis, piles and skin diseases, etc.

In this regard, our present study has been selected three medicinal plants, seeds in *Nigella sativa* & *Entada pursaetha* and fruits in *Ficus glomerata*. From the formulation of *AgNPs Liv-Pro-08* has been formed by

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combining in specific ratio of these selected samples. The aim of present research is to highlight the Phytochemical investigation and antimicrobial analysis carried out on the green synthesized polyherbal formulation so that more pharmacological studies could be conducted to investigate the unexploited potential.

II.MATERIALS AND METHODS

2.1 Collection of plant samples

Nigella sativa and Entada pursaetha seeds and Ficus glomerata fruits were collected from kolli hills, Namakkal district, Tamil Nadu, India.

2.2 Processing of plant sample

The fruits and seeds of *Nigella sativa and Entada pursaetha seeds and Ficus glomerata* collected were dried under shade and then ground into fine powder form (80 mesh sieve size) by electrical grinder. Powdered sample stored in clean paper bags.

2.3 Preparation of aqueous extract from Liv-Pro-08 polyherbal formulation

The aqueous extract of Liv-Pro-08 polyherbal formulation prepared by taking respective ratio of 10 g of powdered samples in 100 ml of distilled water for 30 minutes. The extracts are then filtered using filter paper or Whatman filter paper. The yield of aqueous extract stored in refrigerator at 4° C for further use.

2.4 Green synthesis of AgNPs from Liv-Pro-08 polyherbal formulation

10ml of Liv-Pro-08 polyherbal formulation extract added to 100 ml of 1 mM aqueous silver nitrate solution in a 250 ml conical flask. The colour of the solution changed to brownish colour when the solution of extract and silver nitrate solution was stirred for homogeneous mixing. The flask was kept at room temperature overnight and the Ag nanoparticles separated out which settled at the bottom of this solution. The nanoparticle thus obtained was purified by repeated centrifugation method at 10,000 rpm for 15 min followed by redispersion of the pellet in deionized water. Later the Ag nanoparticles were dried in an oven at 80°C. The synthesized Ag nanoparticles were characterized by pH of solution, UVvisible, FTIR and XRD.

2.5 phytochemical screening

Qualitative analysis of Secondary metabolites

2.5.1 Test for Alkaloids

Dragendroff's test: The extract is treated with few drops of dragendroff's reagent. The Orange brown precipitate coloration is observed.

2.5.2 Test for Flavanoids

Wolf am test for Isoflavanoids: The extract is treated with sodium amalgam and con.HCl. The pink colour formation is observed.

2.5.3 Test for Phenolic compounds

Ferric chloride test: The extract is treated with 2ml of water and 10% aqueous ferric chloride solution. The Blue or green coloration is observed.

2.5.4 Test for Tannins

lead acetate test: The extract is treated with few drops of 1% lead acetate solution. The Yellow or red precipitate formation is observed.

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2.5.5 Test for Terpenoids: The extract is treated with 2ml of chloroform and 1ml of conc. H2SO4. The reddish brown color formation is observed. The Ethanolic extract is treated with 1ml of 2, 4- dinitrophenyl hydrazine in 2M HCl. The Yellow orange color formation is observed.

25.6 Test for lignin: The extract add with phloroglucinol and 1 ml of conc.HCl.red colour formation is observed

2.5.7 Test for saponins: The extracts add with sodium bicarbonate and shake well. honey comb froth formation is observed.

2.5.7 Test for steroids: The extract added with 2ml of chloroform. And conc.sulphuric acid added to the sides of the test tube.

2.5.8 Test for Glycoside

Glycoside test: 0.5 mg of extract was dissolved in 1 ml of water and then aqueous NaOH solution was added. Formation of yellow color indicates the presence of glycosides.

2.5.9 Test for Carbohydrates

Fehling's test : Five ml of Fehling's solution was added to 0.5 mg of extract and boiled in a water bath. The formation of yellow or red precipitate indicates the presence of reducing power.

2.5.10 Test for Protein & amino acids

Biuret test: To 0.5 mg of extract equal volume of 40% NaOH solution and two drops of 1% copper sulphate solution was added. The appearance of violet colour indicates the presence of protein.

2.6 Selected test microorganisms

Extracts were tested against pathogenic microbes, including the bacteria *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa*; the fungi *Aspergillus niger* and *Aspergillus fumigatus.*

2.6.1 Antibacterial Assay

Antibacterial activity of plant extracts was carried using cup-plate agar diffusion method with some minor modifications. [22]

2.7 Antifungal Assay

The cup-plate agar diffusion method was adopted with some minor modifications to assess the antifungal activity of prepared extracts..

III. RESULTS AND DISCUSSION

With the increase in the incidence of resistance to antibiotics, alternative natural products of plants could be of interest. Some plant extracts and phytochemicals are known to have antimicrobial properties, which could be of great importance in the therapeutic treatments[17]. The preliminary phytochemical results of green synthesized polyherbal formulation were showed in the table - 1. Phytochemical screening of AgNPs Liv-Pro-08 contained Glycosides, phenols and tannins. It has been mentioned that antioxidant activity of plants might be due to their phenolic compounds[18]. Flavonoids are most commonly known for their antioxidant activity. They are modifiers which modify the body's reactions to allergens, viruses, and carcinogens. They show anti-allergic, anti-inflammatory, antimicrobial and anticancer activity. The presence of alkaloids explains its anti-bacterial

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activity, since this phytochemical is reported to have anti-bacterial activity[19]. Tannins are reported to have various physiological effects like anti-irritant, antisecretolytic, antiphlogistic, antimicrobial and antiparasitic effects[20]. Phytotherapeutically tannin-containing plants are used to treat nonspecific diarrhoea, inflammations of mouth and throat and slightly injured skins[21].

The antimicrobial activity have been screened because of its great medicinal relevance with the recent years, infections have increased to a great extent and resistance against antibiotics, become an ever increasing therapeutic problem. Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobial compounds[22]

The table 2 represents the effect of silver nanoparticles synthesized using AgNPs Liv-Pro-08 extract on selected strains of bacteria is comparative standard. The zone of inhibition developed by the nanoparticles with nigella sativa extract of various concentration (25,50,75,µl). The results of the antibacterial screening by cup diffusion method using different bacterial strains in different volume of AgNps of nigella sativa is given below. Table – 2 represents the antibacterial effect of selected extracts of AgNPs Liv-Pro-08 by cup diffusion method against selected bacterial strains and the zone of inhibition was assessed in millimetre diameter. The extract of AgNPs Liv-Pro-08 showed moderate zone of inhibition against *Klebsiella pneumonia followed by Escherichia coli* and minimal response against *Pseudomonas aeruginosa*.

The different volume of AgNps of *Liv-Pro-08* was used to screen for its antifungal effect. Table-3 results obtained clearly states that the sample posses antifungal effect against the selected fungal strains. The results of antifungal screening by cup diffusion method using different fungal strains

IV.TABLES

Table 1: Preliminary phytochemical screening of AgNPs Liv-Pro-08 polyherbal formulation

Parameter	AgNPs Liv-Pro-08 extract
Alkaloids	+
Flavonoids	+
Saponins	+
Terpenoids	-
Tannins	+
Cardiac glycoside	+
Phototannis	-
Carbohydrate	+
Protein	+

"+" indicates presence, " - " indicates absence.

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Table 2: Antibacterial activity of AgNps from Liv-Pro-08 polyherbal formulation

S.NO	Name of the microorganism	Zone of Inhibition of Tetracycline (50 µl)	Zone of inhibition in Different volume of AgNPs Liv-Pro-08 polyherbal formulation in mm (Concentration 50mg/ml)		
			25 µl	50 µl	75 µl
1.	***Escherichia Coli***	5	0.2	0.3	0.4
2.	***Pseudomonas aeruginosa***	7	0.3	0.4	0.6
3.	**Klebseilla pneumoniae**	9	0.5	0.6	0.8

Table 3 Antifungal activity of AgNps of Liv-Pro-08 polyherbal formulation

S.NO	Name of the microorganism	Zone of inhibition of quardiline (50 µl)	Zone of inhibition in Different volume of AgNPsLiv-Pro-08 polyherbal formulation in mm (Concentration 50mg/ml)		
			25 µl	**50 µl**	**75 µl**
1.	**Aspergillus niger**	**2**	0.2	0.5	0.8
2.	**Fusarium oxysporum**	**1**	0.2	0.3	0.4

V. CONCLUSION

The exploitation of various biomaterials for the biosynthesis of nanoparticles is considered as green technology as it does not involve any harmful effects. The present study reports that the synthesis of silver nanoparticles and its powerful antimicrobial activity of synthesized silver nanoparticles from the Liv-Pro-08 polyherbal formulation extract may be due to the presence of various phytochemical constituents in them .Therefore,

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extracts of nanobased Liv-Pro-08 polyherbal formulation could be recommended as source of pharmaceutical materials required for the preparation of new antimicrobial agents.

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