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PREPARATION OF NANOPRATICLES OF ACTIVE SECONDARY METABOLITES FROM BRAYOPHYLLUM PINNATUM ANTIBACTERIAL ANALYSIS

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ABSTRACT

Bryophyllum pinnatum is the good source for the medicinal drug and have the properties of anti-diabetic, antipyretic etc. *Bryophyllum pinnatum* has been used to the medicines due to their antiviral, antimicrobial, anticancer, and anti-inflammatory properties. In order to find out the most effective solvent for extraction of biologically active compounds antibiogram analysis has been done by using 80% methanol, ethyl acetate, acetone, benzene extract. 80% methanol was concluded as a most suitable solvent by giving the maximum ZOI against, *E. coli* (14.6 mm), *S. aureus* (16.5 mm) *and P. aeruginosa* (17 mm). Therefore the nano-conjugates of the plant extract have been examined in the laboratory. The nano conjugates were prepared by sing the Cooper nano paowder and crude organic extract of the *Bryophyllum pinnatum* leaf in the ratio of the 1:1, 1:2, 2:1. These nano conjugates showed maximum activity as compare to the normal crude extract of *Bryophyllum pinnatum* leaf.

Key words: *Bryophyllum pinnatum,* Nano-conjugates, Anti-inflammatory, Alkaloids, Flavonoids.

INTRODUCTION

Medical herbalism is the practice of healing with medicinal plants **[1]**. Modern western treatment is different from medical herbalism, but at some point these two merge. For example, the use of friar's balsam or benzoin tincture for treating colds, the use of aloe vera gel for treating sunburn and bruises and the use of cascara or senna to relieve constipation **[2]**. The tendency in modern medicine is to use synthetic drugs that eventually were modeled on compounds obtained mainly from plants. Therefore, whether the plants are used as a whole, or extracts or their synthetics, their discovery originated from the long term practice of medical herbalism by Man **[3]**. Medicinal plants are those plants which are rich in secondary - metabolites and arc potential source of drugs. These secondary metabolites include alkaloids, glycosides, coumarins, flavonoids, steroids etc **[4,5]**.India is one of the few countries where almost all the known medicinal plants can be cultivated in some part of the country or the other **[6]**. Among various plants there is great demand in the country and abroad are as opium poppy, tropane alkaloid bearing plants, sapogenin bearing yams, senna, cinchona and ipecae **[7,8]**. The ancient Indian system of medicine is mainly plant based matesia medica making use of most of our native plants **[9]**. It catres the needs of rural population of our country. India has about 2,000 species of medicinal plants and a vast geographical area with high production potential and varied agroclirnatical conditions **[10]**. Some of the plats which are known and unknown in nature for their medicinal properties are: *Catharanthus roseus* (Vinca) also known as the Madagascar periwinkle or rosy periwinkle or Sadabahar is grown as an ornamental plant in the garden. The pink and the white varieties are grown for its medicinal value in Ayurveda, the plant root and shoot are poisonous, yet used as medicine against several diseases **[11,12]**. *Bryophyllum pinnatum,* also known as Miracle Leaf, Katakataka, Life Plant and Pashan Bheda / Patharchur, is a succulent perennial herb. *Bryophyllum pinnatum* is given for the treatment of a cough, asthma, cold with candy sugar **[13]**. *Solanum nigrum* is used for both culinary and medicinal purposes. The fruits grow in bunches. The leaves, stem and fruit are used for various ailments **[14]**.

Ficus racemosa is a species of plant in the family Moraceae. Popularly known as the cluster fig tree, Indian fig tree or goolar (gular). In countries like India, the bark is rubbed on a stone with water to make a paste and the paste is applied over the skin which is afflicted by boils or mosquito bites **[15, 16]**. The herb *Butea Monosperma* belongs to the plant family Fabaceae and the order Fabales. This herb is commonly known as Palas in Hindi. The gum of the tree can be used to relieve Diarrhea when taken in three doses regularly **[17]**. The secondary metabolites of such medicinal plant are in use from ancient times **[18]**. The extracts can be used in the form of nanoparticles or nanoconjugates to enhance the activity of the metabolites **[19]**. Nanotechnology is expected to be the basis of many main technological innovations in the 21st century. Research and development in this field is growing rapidly throughout the world. A major output of this activity is the development of new materials in the nanometer scale, including nanoparticles **[20]**. N.K. Udayaprakash *et al;* (2013) reported that the synthesis of nanoparticles of a particular plant extracts were being widely reported nowadays. The key principle behind the synthesis process is considered to be the presence of proteins based compound like Metallothionins and Phytochelators. The Secondary metabolites like phenols, Tannins and Flavonoids are found to play a major role in the formation of nano sized particles **[21]**. Naheed Ahmed and Sheena sharma (2012) reported that the biosynthesis of nanoparticles are cost effective and environmental friendly method. The study shows the synthesis of silver nanoparticles using extract of *Ananas comosus* reducing aqueous silver nitrate. The nanoparticles are characterized by UV- Visible spectroscopy, XDR, FTIR, AFM, FESEM and EDX **[22]**.

METHODOLOGY

Sample collection: the pant leaves samples were collected from the herbal nursery located in Aliganj, Lucknow for the work.

Extraction of active metabolites: the samples were dried after washing and then converted to powder. The samples were dipped into polar and non-polar solvents in 1:10 ratio and then kept for48 hours. The solvents were evaporated after filtering the metabolites, and then these metabolites were scratched in DMSO and preserved for screening. Filtered metabolites were also preserved for the preparation of nano-conjugates **[23]**.

Antibiogram analysis: the extracts were screened for antibacterial properties by using agar well diffusion method and the results were observed by calculating the zone of inhibition **[24]**.

Phytochemical analysis: Different activities possessed by plant in due to the presence of certain bioactive components or the secondary metabolites. Trease and Evans *et al*., 1989, gave a standard procedure for the identification of these secondary metabolites **[25]**.

Test for Terpinoids (Salkowiski test): 1ml of the extract was mixed with 2ml of chloroform and concentrated H2SO4 (3ml) was carefully added to form a layer. A reddish brown colouration of the interface was formed to indicate positive results for the presence of terpinoids .

Test for Flavonoids: 1ml of extract was dissolved in diluted NaOH and HCl was added. A yellow solution that turns colourless, indicate the presence of flavonoids.

Test for Tannins: 1ml of extract was added to few drops of 1% lead acetate. A yellowish precipitate indicate the presence of tannins.

Test for Steroids: 1ml of the extract was dissolved in 10ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tubes. The upper layer turns red and sulphuric acid layer showed yellow with green flurosence. This indicate the presence of steroids.

Test for Saponins: Add 1ml of distilled water to 0.1ml of extract and shake vigorously and observe for persistent froth. Mix 3 drops of Olive oil. Formation of emulsion indicates the presence of Saponins.

Test for Glycosides: 0.1ml of extract was dissolved in 1ml of glacial acetic acid containing 1 drop of ferric chloride solution. This was under layered with 0.1 ml of concentrated sulphuric acid. Presence of brown ring at the interface indicates the presence of Glycosides.

Test for Alkaloids: Stir 3g of extract with ethanol containing 3% tartaric acid. The filtrate shared into 3 breakers and tested for alkaloids as follows:

In the first beaker, add Hagar's Reagent, and in the second beaker add Mayer's reagent and in the third beaker add Marquin's reagent. Precipitation in any of the three test tube indicates the presence of Alkaloids.

Preparation of copper nanoparticles: 35ml of 62.7M $CuSO_4$ was prepared and then $CuSO_4$ solution was added to the ascorbic acid. Dark green solution formed and left for 30 mins. Further orange colour metallic substance starts deposits on wall of the beaker.

The solution can be left for few days for the reaction but we prefer to accelerate the process. The solution was boiled and 4-5 cycles will result in a reasonable copper deposit.

The concentration of the solution is lowered enough so that the subsequent reaction is only very slow. The solution was filtered and then washing was carried out by using absolute ethanol. Hence the 48% yield Nano Copper powder was obtained on starting copper sulphate **[26]**.

Table 1: For the preparation of suspension plant extract copper nanoparticle we select the following criteria in the different ratios such as:-

S.no	Ratio	Concentration of plant extract and copp nanoparticle		Solvent use	Incubation tir
		Copper	Plant extract	DMSO	
1.	(1:1)	1mg	1mg	1ml	24 hours
2.	(1:2)	1 mg	2 mg	1ml	24 hours
3.	(2:1)	2 mg	1 mg	1ml	24 hours

RESULTS:

Plants samples

1. Catheranthus roseus (Sadabahar)

2. ***Brayophyllum pinnatum*** (Ajooba)

3.Solanum Nigirum (Makoi)

4. Butea Monosperma (Dhak or Palash)

5. ***Ficus Racemosa*** (Gular)

Figure 1: collected leaf samples of plants

Antibiogram analysis of plant extract

Table 2: Antibiogram analysis of Acetone, Benzene, Ethyl Acetate and 80% methanol of Brayophyllum pinnatum leaf

Pathogens	Zone of Inhibition (mm)			
	Acetone	**Benzene**	**Ethyl acetate**	**Methanol 80%**
E. coli	14.6	10	11.5	12.5
P. aeruginosa	16.5	19	12	12.5
S. aureus	17	19	12	12.5

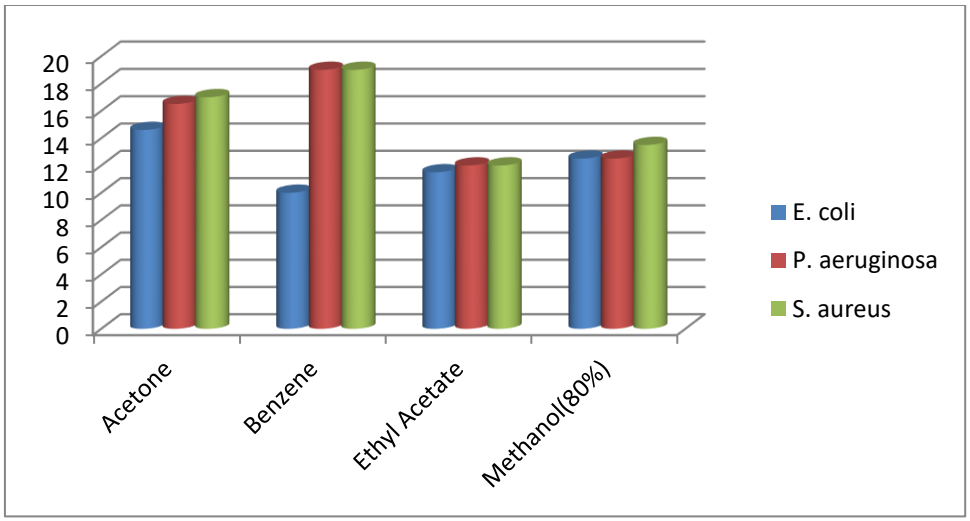

Figure 2: Graph representing the antibiogram analysis of Acetone, Benzene, Ethyl Acetate and 80% methanol of Brayophyllum pinnatum leaf

Table 3: Antibiogram analysis of Acetone, Hexane, Choloroform and 80% methanol of Catheranthus roseus leaf

Pathogens	Zone of Inhibition (mm)			
	Acetone	**Benzene**	**Ethyl acetate**	**Methanol 80%**
E. coli	17	15.5	19	16.5
P. aeruginosa	0	0	0	0
S. aureus	0	0	0	0

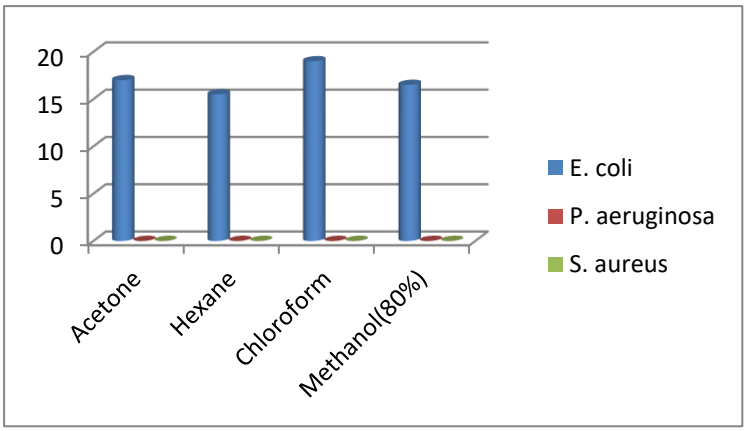

Figure 3: Graph representing the antibiogram analysis of Acetone, Hexane, Chloroform and 80% methanol of *Catheranthus roseus* leaf

Table 4: Antibiogram analysis of Acetone, Benzene, Ethyl Acetate and 80% methanol of *Monosperma Butea* leaf

Pathogens	Zone of Inhibition (mm)			
	Acetone	Benzene	Ethyl acetate	Methanol 80%
E. coli	11	0	11.5	10.5
P. aeruginosa	12	14.5	11.5	12.5
S. aureus	10.5	0	11.5	12.5

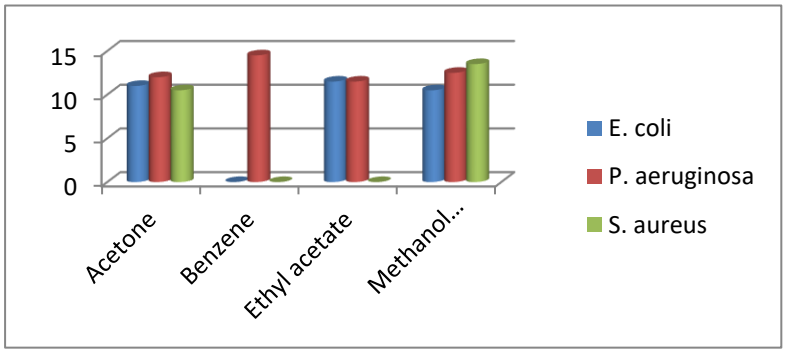

Figure 4 : Graph representing the antibiogram analysis of Acetone, Benzene, Ethyl Acetate and 80% methanol of Monosperma Butea leaf

Table 5: Antibiogram analysis of Acetone, Benzene, Ethyl Acetate and 80% methanol of Solanum Nigirum leaf.

Pathogen	Zone of Inhibition (mm)			
	Acetor	Benzer	Ethyl acetat	Methan 80%
E. coli	12.5	0	13	0
P. aerugino	12.5	13	11.5	0
S. aureu	0	0	0	0

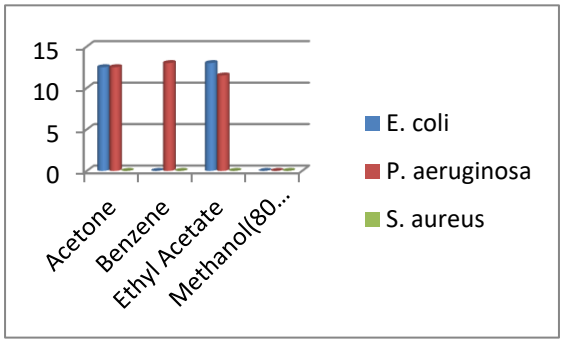

Figure 5: Graph representing the antibiogram analysis of Acetone, Benzene, Ethyl Acetate and 80% methanol of *Solanum nigirum* leaf.

Table 6: Antibiogram analysis of Acetone, Benzene, Ethyl Acetate and 80% methanol of *Ficus racemosa* leaf

Pathogen	Zone of Inhibition (mm)			
	Acetor	Benzer	Ethyl acetat	Methar 80%
E. coli	0	0	12	16.5
P. aerugino	0	0	11.5	18
S. aureu	0	0	0	13

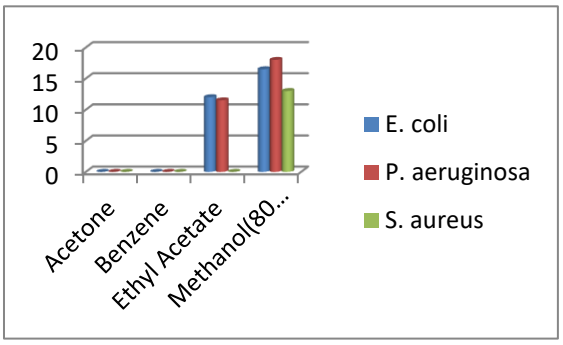

Figure 6: Graph representing the antibiogram analysis of Acetone, Benzene, Ethyl Acetate and 80% methanol of *Ficus racemosa* leaf

Screening of copper nano-conjugates with *Brayophyllum pinnatum* leaf extract

Table 7: Antibiogram analysis of methanol 80%, Acetone, ethyl acetate, benzene leaf extract *Brayophyllum pinnatum* + copper nanoparticles *against Escherichia coli (E. coli), Staphylococcus aureus (S. aureus)* and *Pseudomonas aeruginosa (P. aeruginosa)*

Pathogens	Zone of Inhibition (mm)				
	1:1	1:2	2:1	NC	PC
Methanol 80%					
E. coli	8	0	0	0	16.5
P. aeruginosa	0	18.5	11.5	0	18
S. aureus	0	19	0	0	13
Ethyl Acetate					
E. coli	0	0	0	0	19
P. aeruginosa	19.5	23	21	0	11
S. aureus	23.5	23	23	0	15
Benzene					
E. coli	0	21.5	0	0	18
P. aeruginosa	20	0	0	0	13
S. aureus	0	0	0	0	14
Acetone					
E. coli	0	0	19.8	0	12
P. aeruginosa	0	0	0	0	14
S. aureus	0	0	0	0	13

Phytochemical analysis:

Table 8: Tabular representation of the phytochemical analysis of the *Butea monosperma* leaf.

S n	Secondary metabolites	Acetone	Benzene	Ethyl acetate	80% methanc
1.	Flavonoids	+	-	-	+
2.	Saponins	-	+	-	+
3.	Steroids	+	+	-	+
4.	Glycosides	-	-	-	+
5.	Carbohydrat	-	+	-	+
6.	Terpenoids	-	+	-	+
7.	Alkaloids	+	-	-	+

Table 9: Tabular representation of the phytochemical analysis of the *Ficus racemosa* leaf.

S n	Secondary metabolites	Acetone	Benzene	Ethyl acetate	80% methanc
1.	Flavonoids	+	-	+	+
2.	Saponins	-	-	-	+
3.	Steroids	-	-	-	+
4.	Glycosides	-	-	-	+
5.	Carbohydrat	-	-	-	-
6.	Terpenoids	-	-	-	+
7.	Alkaloids	+	-	-	+

Table 10: Tabular representation of the phytochemical analysis of the *Solanum nigirum* Leaf.

S n	Secondary metabolites	Acetone	Benzene	Ethyl acetate	80% methanc
1.	Flavonoids	-	-	-	+
2.	Saponins	-	+	-	+
3.	Steroids	+	+	+	+
4.	Glycosides	-	-	-	+
5.	Carbohydrat	+	+	+	+
6.	Terpenoids	+	-	-	+
7.	Alkaloids	-	-	+	-

Table 11: Tabular representation of the phytochemical analysis of the *Brayophyllum pinnatum* leaf.

S no	Secondary metabolites	Acetone	Benze	Eth ace e	80% methanc
1.	Flavonoids	+	-	-	+
2.	Saponins	-	-	-	-
3.	Steroids	+	+	+	+
4.	Glycosides	+	-	-	+
5.	Carbohydrates	+	+	+	+
6.	Terpenoids	+	-	+	+
7.	Alkaloids	-	-	-	-

Table 12: Tabular representation of the phytochemical analysis of the *Catheranthus roseus* leaf.

S no	Secondary metabolites	Acetone	Benzene	Ethyl acetate	80% methanc
1.	Flavonoids	+	+	-	-
2.	Saponins	+	+	-	-
3.	Steroids	+	+	+	+
4.	Glycosides	+	+	+	+
5.	Carbohydrat	+	+	+	+
6.	Terpenoids	+	+	-	-
7.	Alkaloids	-	-	-	-

DISCUSSION

With the modernization of the world and advancement in technology we have a great increase in medicines. Nanoparticles (NPs) have various applications in biomedicine and drug delivery carriers and also are widely used in cosmetics. However, the preparation of biocompatible and non-toxic nanomaterials is a very important issue as most of the starting materials are synthesized using toxic chemical reagents. This review introduces the preparation of biocompatible NPs in a range of their concentrations using phytochemicals for biomedicine and biotechnology. And entering in this gateway I choose the number of wild plants in which some are the known plants and some are unknown. The plants which didn't give the good result is *Butea monosperma, Ficus racemosa*, *Solanum nigirum*. On the basis of their best results observation, the two plants *Bryophyllum pinnatum* and *Catheranthus roseus* was selected for the preparation of the nanoparticles with the copper nanoconjugate and their antibiogram analysis for the evaluation of its antimicrobial activity.

The best result of acetone, ethyl acetate, benzene and 80% methanol leaf extract of *Bryophyllum pinnatum* shows the following zone of inhibition against *E. coli, P. aeruginosa, S. aureus,* are 16mm, 19mm, 12mm, 13.5mm and the best results of suspension copper nanoparticles of the acetone, ethyl acetate, benzene and 80% methanol leaf extract of *Bryophyllum pinnatum* in the ratio of (1:1), (1:2), (2:1) shows the following zone of inhibition against *E. coli, P. aeruginosa, S. aureus* are 17.5mm, 20.5mm, 23.5mm, 18.5mm

CONCLUSION

Based on above research work it can be concluded that *Bryophyllum pinnatum* and *Catheranthus roseus* can be the good source for the medicinal drug and can be explored further with the nanoparticles in order to increase the antimicrobial effect.

Further work also includes the further purification of the metabolites responsible for antibacterial properties using sophisticated purification procedure, and the nanoparticle preparation. Pharmacologically evaluation of extracts with the nanoparticles of the various metal ions, for human consumption and also investigation of Phytochemical responsible for antibacterial properties.

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