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Screening of Anti-Microbial Activity of Sahadevi (*Vernonia Cinerea*) by using Norfloxacin as Standard

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Abstract:- The plant Vernonia cinerea of the family Asteraceae is widely distributed in the most tropical and subtropical part of the India. It is commonly known as 'Sahadevi'. According to Ayurvedic pharmacopoeia of India it is recommended for the intermittent fever, blisters, pain, diuresis, various gastro-intestinal disorder, boils, malaria, vaginal discharge and in case of psychoneurosis. Therefore, the present study was designed to evaluate the antimicrobial activity as well as to describe the presence of phytoconstituents through phytochemical screening of the hydro-alcoholic extract of the whole parts of the plant. We carried out the phytochemical screening of the Vernonia cinerea which shows the presence of carbohydrate, reducing and non-reducing sugar, glycoside, phytosterol & terpenoids, phenolic compound & tannin, flavonoids. Antimicrobial activity of the Vernonia cinerea against most dominating microbes like Staphylococcus aureus, Escherichia coli, Proteus mirabilis, Candida Spp. (albicans) through well diffusion method. The hydro-alcoholic extract at various concentrations produces significant antimicrobial and antifungal activity against the selected microorganism when compared with standard drug Norfloxacin for antibacterial and antifungal activity.

Keyword:- *Vernonia cinerea, Hydro-alcoholic extract, Phytochemical screening, Anti-microbial activity.*

I. INTRODUCTION

Infectious diseases have been consistently found to be among the leading causes of threat to global health. The World Health Organization (WHO) in 2013 reported that infectious diseases accounted for 61.7% (5.9 million) of the 9.6 million deaths in the sub-Saharan African region. Plants with medicinal value have found application in healthcare from the golden years. Globally, there are evidence-based studies to verify the efficacy of medicinal plants, and some of these shreds of evidence have provided insights into the synthesis of plant-based compounds with therapeutic application [1]. The plant *Vernonia cinerea* belongs to family Asteraceae. It is commonly known as Sahadevi in Hindi, Kukasim in Bengali, Little ironweed in English, is an annual herb found in upland crop area, waste places and roadsides [2]. *Vernonia cinerea* (Asteraceae), an annual herb, is reported to have many medicinal properties. *Vernonia cinerea* has many therapeutic uses in different traditional medicines of the world, including use for treatment of a number of disorders such as malaria fever, worms, pain, inflammation, infections, diuresis, cancer, abortion, and various gastrointestinal disorders. Every part of the plant can be used medicinally. The leaves are eaten as a potherb. Fresh juice of the leaves is given in amoebiasis. A poultice of the leaves is used against humid herpes, eczema, ring worm and for the extraction of Guinea worm [3]. Root is biter and used as an anthelmintic and given in diarrhea and stomachache. The flowers are used in conjunctivitis and fever [4]. The seeds are commonly used as an anthelmintic and effective against roundworms and threadworms. They are useful for leucoderma, psoriasis and other chronic skin diseases [5]. An extract of the plant is used to relieve cold and menstruation-related problems. The plant has also been used as a tonic, stomachic, and astringent. The use of different parts of kukasim against different worms and microorganisms suggest that the whole plant can produce significant antimicrobial activity. Thus we have selected this plant for screening antibacterial and antifungal activities against various gram (+), gram (-) and fugal microorganisms. Whole plant gave triterpene compounds betaamyrin acetate, lupeol acetate, betaamyrinandlupeol; sterols- beta- sitosterol, stigmasterol and alpha-spinasterol; phenolic resin and potassium chloride. Parts that were used include the flower (treatment of conjunctivitis), seeds (used as anthelmintic), root (dropsy), juice (piles). The whole plant is also considered to promote perspiration in the febrile condition. The plant is anthelmintic, antibacterial, antiviral, antifungal, anti-inflammatory, diuretic, and stomachic. The roots are useful in diarrhoea, cough, inflammations, skin diseases, leprosy, renal and vesicle calculi. The leaves are useful in humid herpes, eczema, ring worm, guinea worms, and elephantiasis [6]. In addition, the ethanolic extract inhibited 53.13% and 62.43% haemolysis of red blood cells in heat-induced and hypotonic solution conditions, respectively as compared to acetylsalicylic acid. The flowers are used in conjunctivitis, vitiated condition of fever. Both polar and non-polar fraction of the plant extract showed analgesic, antipyretic and anti-inflammatory effect. Polar extract of *Vernonia cinerea* is found to have anti-diarrhoeal activity but there is no study.

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II. METHODS AND MATERIALS

A. Plant collection

The plant of *Vernonia cinerea* was collected from the locality of Haldia from the district East Medinipur, West Bengal. Firstly the plant was identified and carefully collected from the neighbouring dump,waste and road side areas**.** To confirm that the plant is *Vernonia cinerea* visual examination were verified with the help of Plantnet app. The final identification of the plant was done by Botanical survey of India (Howarh- 711103, W.B.). After collecting the plant *Vernonia Cinerea* the unwanted plant impurities were separated out and our plant of interest were pulled out. After this, the leaves, steam, flower, and roots were isolated and separately shade dried.

B. Preparation of plant extracts

The dried plants were grinded in the form of powder in the ratio of 50:50 (course powder: fine powder) with the help of mechanical grinder. About 30 grams of whole plant powdered were taken in a beaker. Exact 280 ml of hydro-alcoholic solvent (70:30 ratios of iso-propyl alcohol and water) was added in to the beaker and covered with aluminium foil for 3 days (72 hours) with frequent agitation.

Filter the extract by using Whatman number-1 filter paper to remove the plant parts. Distillation method has used to remove the alcohol from the extract. Then alcohol free extract made semisolid compound by double boiler method. This semisolid extract was stored in clean, dry, sterile, labelled glass vials.

C. Bacterial sample

a) Escherachia coli

Escherachia coli are gram negative and rod shaped bacteria. The gastrointestinal tract of warm-blooded animals is colonized by E. coli within a couple of hours or a few days after birth. But E. coli is more than a harmless intestinal inhabitant; it can also be a highly versatile, and frequently deadly, pathogen. It is responsible for several intestinal and extraintestinal diseases. ExPEC (Extraintestinal Pathogenic Escherichia coli) strains are the cause of a diverse spectrum of invasive human and animal infections, often leading to septicemia. Uropathogenic E. coli (UPEC) can cause urinary tract infections. EIEC (Enteroinvasive E. coli) causes a broad spectrum of human's diseases like invasive inflammatory colitis but may also elicit a watery diarrhoea syndrome [7]. E. coli is usually sensitive to ciprofloxacin, Bactrim or Gentamycin. In most cases treating with antibiotics can triple the risk of developing hemolytic uremic syndrome leading to kidney failure [8].

b) Proteus mirabilis

Proteus mirabilis is a Gram-nagative, facultatively anaerobic, rod-shaped bacterium. In liquid culture, p.mirabilis has a short shape and typically processes a few of the peritrichous flagella [9]. However on rich solid media, P.mirabilis differentiate into very long typically 20-80 µm, although cells longer than 100 µm occur, nonseptate polyploid cell with hundreds to thousands of flagella. P. marabilis is mostly noted for its swarming motility and urease activity, frequently associated with infection of the urinary tract, especially in the complicated or catheter-associated urinary tract infection [10]. This rod shape bacterium has the ability to produce high level of urease, which hydrolyzes urea to ammonia, so make the urine more alkaline. It remain untreated, the increase alkalinity can lead to formation of crystals of struvite, calcium carbonate or apatite, which can result in kidney stone [11]. P.mirabilis is generally susceptible to most antibiotic apart from tetracycline and nitrofurantion, but 10-20% of P.mirabilis strains are also resistant to first-generation cephalosporins and ampicillin [12, 13].

c) Staphylococcus aureus

Staphylococci are gram-positive bacteria of 0.5 to 1.5 µm diameters distinguished by individual cocci, separated into clusters with grape-like in more than one plane [14]. They are non-motile, non-spore forming facultative anaerobes. S. aureus is a significant pathogen that infects both hospitalized and stable immunological patients. It may cause local skin, nose, urethra, vagina and gastrointestinal tract infections, which are often mild and do not endanger lives [15]. Enterotoxin ingestion from S. aureus can cause food poisoning in contaminated food [16]. Staphylococci are strongly resistant to the most widely used antibiotics, such as erythromycin, ampicillin b and tetracycline. Vancomycin is inhibiting S. aureus production in some cases [17].

d) Candida Spp.

Candida is a genus of yeasts and is the most common cause of fungal infections worldwide it belongs to the family Saccharomycetaceae. The most commonly used type of fungus is candida albicans, candida is commonly leaves on skin inside the body such as the mouth, throat, gut, and vagina without causing any problem [18].

D. Culturing of bacterial samples

Pure ATCC bacterial strain of Escherichia coli, Proteus mirabilis, Staphylococcus aureus, Candida Spp were obtained from Dr. B.C Roy Hospital, ICARE, Bamdishnupur, Balughata road, Haldia, West Bengal. The culture was obtained on Agar media. Each of the bacteria strain was again sub cultured on agar media for experimental work.

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a) Preparation of Agar media for sub-culturing:

MATERIALS	AMOUNTS
1. Agar Agar	4 gm
2. Sodium chloride (Nacl)	0.5 gm
3. Beef Extact	0.3 gm
4. Peptone	0.5 gm

Table 1: Preparation of agar media for preparing 100 ml of agar media

b) Assessment of anti-microbial assay using well diffusion method:
16g of Agar with 2 gm of NaCl, 1.2 gm of beef extract and 2 gm of peptone were dissolved in 400 ml of distilled water. With frequent agitation the media was boiled for one minute to completely dissolve all the ingredients together and it was autoclaved at 121°C for 15 minutes and later cooled to room temperature (The final pH was maintained at 7.3 ± 0.1 at 25°C). Cooled Agar media was poured into clean, sterile petri dishes on a level, horizontal surface to give uniform depth. This was performed inside the Laminar Airflow Chamber. The plates were allowed to cool at room temperature. After solidification of the media, 1 ml of bacterial solution were inoculated into the petri plates using sterile, autoclaved ear buds or L shaped Glass rod and spread uniformly. Four wells were punched on each of the plates containing specific concentration of standard and extract [19, 20].

c) Standard preparation
400 mg of Norfloxacin was dissolved in 100 ml of distilled water to prepare a stock solution. Taken 2.5 ml of stock solution and dilute with distilled to make up the volume up to 100 ml to prepare work solution of 0.1 mg/ml. To prepare standard work solution of 0.01 mg/ml and 0.02 mg/ml concentration taken 0.1 ml and 0.2 ml of prepared solution respectively and make up the volume 10 ml with distilled water.

d) Sample Preparation
150 mg of *Vernonia cinerea* extract was dissolved in 15 ml of hydroalcoholic solvent to prepared 10 mg/ml of sample stock solution. Take 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml, 3.0 ml and 3.5 ml of Stock solution and pour into 10 ml volumetric flask separately and make up the volume up to 100 ml with distilled water to prepare standard solution of 1mg/ml, 1.5 mg/ml, 2.0 mg/ml, 2.5 mg/ml, 3.0 mg/ml and 3.5 mg/ml respectively.

Well No.	Concentration of std. (Norfloxacin) (mg/ml)	Concentration of Extract (*Vernonia cinerea*) (mg/ml)
1.	0.01	-
2.	-	1.0
3.	-	1.5
4.	-	2.0

Table 2: Distribution of sampling of standard and extract in Plate 1

Well No.	Concentration of std. (Norfloxacin) (mg/ml)	Concentration of Extract (*Vernonia cinerea*) (mg/ml)
1.	0.02	-
2.	-	2.5
3.	-	3.0
4.	-	3.5

Table 3: Distribution of sampling of standard and extract in Plate 2

The plates were incubated at 37°C in the incubator for 24 hours. Results were observed, diameter of the Clearing zones was noted down, photographed and the data was tabulated.

III. RESULT AND DISCUSSION

Various parts of the herb *vernonia cinerea* reported in a wide range of therapeutic applications such as microbial infections, worm infections, malaria, inflammation, diuretics. In this study the hydro alcoholic extract of whole part of *vernonia cinerea* was evaluated for antimicrobial activity against most dominating microbes like *Staphylococcus aureus, Escherichia coli, Proteus mirabilis, Candida Spp. (albicans)* through well diffusion method.

The hydro-alcoholic extract of herb *vernonia cinerea* contains a large number of metabolites were screened by phytochemical investigation. The study showed presence of carbohydrates and some secondary metabolites *viz.* saponins, phenolic compounds, tannins and flavonoid which could be responsible for therapeutic activity of plant uses in traditional medicines.

Constituents	Tests	Results
Carbohydrate	Molisch Test	Carbohydrate is Present
	Benedict Test	Reducing sugar is Present
	Fehling's Test	Reducing sugar is Present
	Barfoed Test	Reducing sugar is Present
Alkaloid	Mayer's Test	Fail
	Wagners's Test	Fail
	Hager's Test	Fail
Saponin	Foam Test	Fail
Phenolic Compound & Tanin	Ferric Chloride Test	Phenolic compound and Tanin is Present
	Lead Acetate Test	Phenolic compound and Tanin is Present
Flavonoid	Shinoda Test	Flavonoid is present

Table 4: The phytochemical screening of the hydro-alcoholic extract of the whole part of *vernonia cinerea*

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In our experimental work, firstly, we have taken amoxicillin as Std. and hydro-alcoholic extract of *Vernonia cinerea* as sample and studied the zone of inhibition. Literature survey explained that amoxicillin has developed resistant towards *Escherishia coli* and other microorganism [21]. Norfloxacin, fluoroquinolone derivatives, a well-established standard is used for experimental work that can work against all four types of microbes that we have used for our antimicrobial study. For experimental work we took Norfloxacin as a standard in two different concentrations of 0.01 mg/ml and 0.02 mg/ml. From the sample extract we took six different concentration of 1mg/ml, 1.5 mg/ml, 2 mg/ml, 2.5 mg/ml, 3 mg/ml and 3.5 mg/ml Result was reported as calculate zone of inhibition areas and did a comparative study on their antimicrobial activity. Zone of inhibition of mentioned concentrations was calculated against four different category of microorganism denoted as GPC, GNB1, GNB2 and Fungus stands for microorganism *staphylococcus aureus, Escherichia coli, Proteus mirabilis* and *Candida spp.* respectively. The extract has been shown to elicit inhibitory action against a broad spectrum gram positive, gram negative and candida when compare with Norfloxacin as standard.

Treatment (Concentration)	**Zone of Inhibition (cm^2)**			
	GPC	**GNB1**	**GNB2**	**Candida**
	***Staphylococcus aureus* (Gram-positive)**	***Escherichia coli* (Gram-negative)**	***Proteus mirabilis* (Gram-negative)**	***Candida spp.* (Fungus)**
Norfloxacin (0.01 mg/ml)	3.46	2.83	2.26	2.54
Norfloxacin (0.02 mg/ml)	3.80	3.26	2.43	3.17

Table 5: Inhibition zone diameters of Norfloxacin standard against various bacteria and fungus

Treatment (Concentration)	**Zone of Inhibition (cm^2)**			
	GPC	**GNB1**	**GNB2**	**Candida**
	***Staphylococcus aureus* (Gram-positive)**	***Escherichia coli* (Gram-negative)**	***Proteus mirabilis* (Gram-negative)**	***Candida spp.* (Fungus)**
Vernonia Cinerea Extract (1.0 mg/ml)	1.53	1.32	1.36	1.36
Vernonia Cinerea Extract (1.5 mg/ml)	1.83	1.62	1.45	1.45
Vernonia Cinerea Extract (2.0 mg/ml)	2.01	1.86	1.60	1.67
Vernonia Cinerea Extract (2.5 mg/ml)	2.08	2.26	1.76	1.81
Vernonia Cinerea Extract (3.0 mg/ml)	2.26	2.83	1.88	1.88
Vernonia Cinerea Extract (3.5 mg/ml)	2.63	3.23	2.06	2.08
Curve Equation	y= 0.196x+1.3707	y= 0.388x+0.8287	y= 0.1414x+1.19	y= 0.1437x+1.2053
Equivalent Concentration (x=y-c/m) mg/ml	**5.82**	**3.07**	**4.28**	**5.14**

Table 6: Inhibition zone diameters of *vernonia cinerea* hydro-alcoholic extract against various bacteria and fungus

In case if GPC (*staphylococcus aureus)*, 5.82 mg/ml of sample (*vernonia cinerea* extract) is equivalent to 0.01 mg/ml of standard Norfloxacin. In case if GNB1 (*Escherichia coli)*, 3.07 mg/ml of sample (*vernonia cinerea* extract) is equivalent to 0.01 mg/ml of standard Norfloxacin. In case if GNB2 (*Proteus mirabilis)*, 4.28 mg/ml of sample (*Vernonia cinerea* extract) is equivalent to 0.01 mg/ml of standard Norfloxacin. In case if Fungus (*Candida spp.*), 5.14 mg/ml of sample (*vernonia cinerea* extract) is equivalent to 0.01 mg/ml of standard Norfloxacin. The study confirms that sample showed significant antimicrobial activity against all microorganisms as compared to Norfloxacin as standard.

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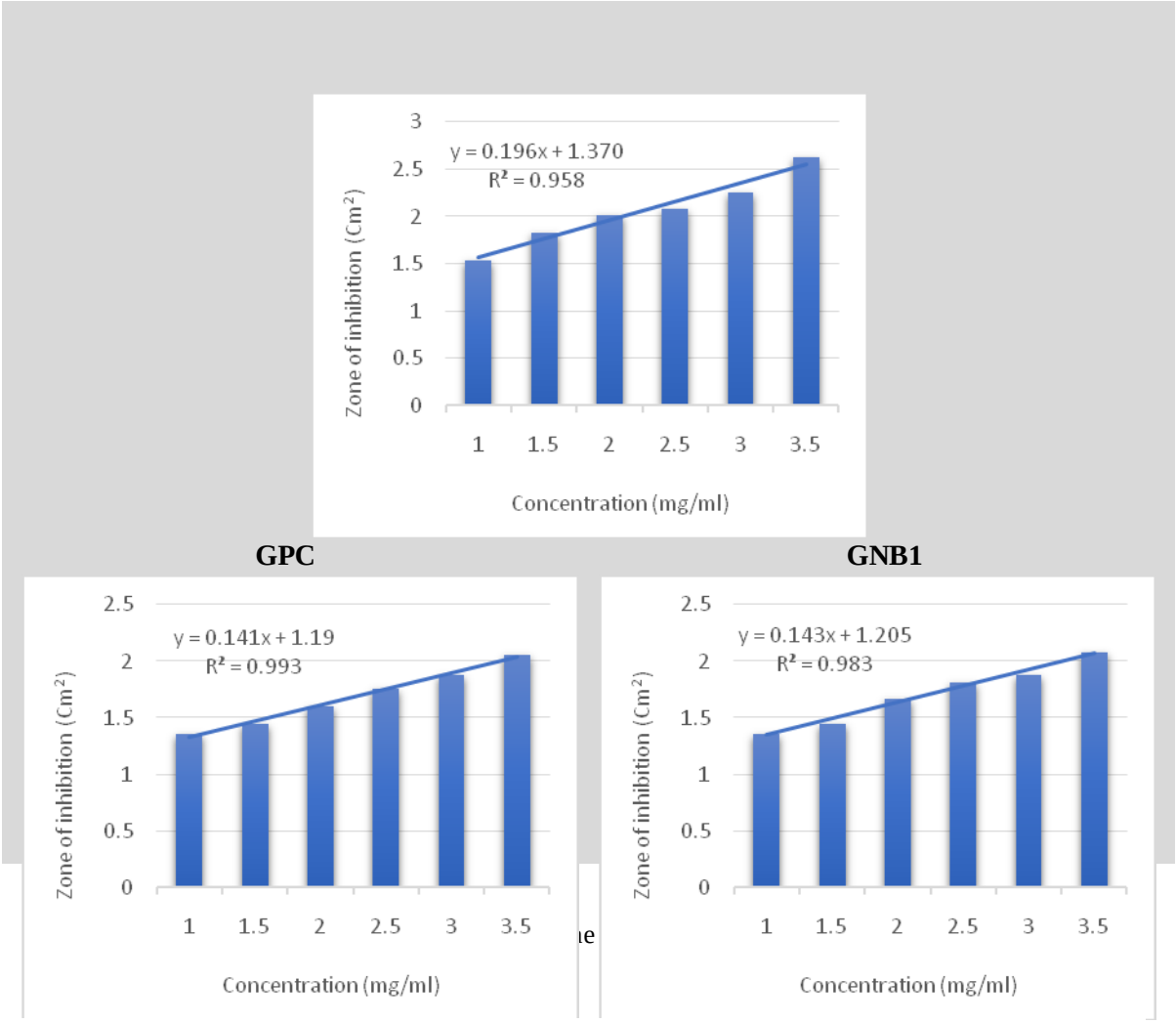

Fig. 2: Zone of inhibition shown by *Vernonia cinerea* extract against different microbes**.**

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IV. CONCLUSION

Study revealed that about 80% of the world's population in developing countries use plant materials as their source of primary health care. Effective extracts could provide potential leads toward the development of novel and environmentally friendly antibacterial agents or lead to standardized phytomedicines. In this study we particularly concentrated on antimicrobial activity of hydroalcoholic extract of *Vernonia cinerea* against two gram-negative bacteria, one gram positive bacteria and *Candida spp*. The present study investigated the antimicrobial activity of hydroalcoholic extract of the plant *V. cinerea* against one gram positive and two-gram negative bacteria and one *candida spp*. and compared with synthetic antimicrobial agent Norfloxacin. The result indicates that the *V. cinerea* extract showed antimicrobial activity against *Proteus mirabils*, *Escherichia coli*, *Staphylococcus aureus* and *Candida spp.* The phytochemical screening revealed the presence of carbohydrate, reducing sugar, glycoside, phytosterol & terpenoids, phenol compound & tannin, flavonoid in the sample extract. The presences of flavonoid give as the hint that the extract of *Vernonia cinerea* has antimicrobial activity. It was observed that Norfloxacin solution and the sample extract showed similar zone of inhibition. The activity was assessed using Multi Drug Resistant strains. Majority of the microbes are pathogenic to human beings.

The hydro-alcoholic extract of *Vernonia cinerea* has been shown to produce significant antimicrobial activity against selective microorganism. In case of GPC, GNB1, GNB2 & *Candida spp.*, the extract having concentration 5.82 mg/ml, 3.07 mg/ml, 4.28 mg/ml, 5.14 mg/ml respectively to showed equivalent result as compared to that of 0.01 Norfloxacin. The result from this study may help to support antimicrobial activity of *Vernonia cinerea* and development of new drugs for the treatment of various diseases. The results obtained from this study confirm antimicrobial activity of *Vernonia cinerea.*

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