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ANTI MICROBIAL AND ANTIOXIDANT EVOLUTION OF AQUEOUS EXTRACT OF ROOTS OF *SWERTIA CHIRATA* USING DISC DIFFUSION, DPPH REGENT METHODS

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ABSTRACT

The biological evolution of of *Swertia chirata* leaves extract, plant belongs to family Combretaceae. The aqueous extract of the plant material was tested for Antimicrobial activity of against Gram positive and Gram negative bacterial strains by observing the zone of inhibition. Antimicrobial activity was done by disc diffusion method at a concentration of, 100,250,500 µg/disc of the extract, using ofloxacin (5µg/disc) as the standard. The bacterial strains used in the study were *Staphycococcus aureus, Bacillus subtilis, Escherichia coli, Proteus vulgaris*, the anti oxidant activity of by using at concentration of 5, 10, 15, 20, 25, 30 µg/ml, using Ascorbic acid as standard. The outcomes of the present study indicated that the aqueous extract of the *Swertia chirata* shows the significance anti bacterial and anti oxidant activity in a concentration of 500 µg/ml, 30µg/ml respectively.

KEY WORDS: *Swertia chirata*, aqueous extract Antibacterial activity, antioxidant activity, DPPH reagent method.

INTRODUCTION

The World Health Organization estimated that 80% of the population of developing countries still relies on traditional medicines, mostly plant drugs for their primary health care needs. Herbs are supposed to be safe but many unsafe and fatal side effects have recently been

reported.[1] Free radicals such as reactive oxygen species are formed during a variety of biochemical reactions and cellular functions. Oxidative stress results from an imbalance between formation and neutralization of pro-oxidants. Free radical formation and the effect of these toxic molecules on cell function are collectively called "oxidative stress." These free radicals are highly reactive, unstable molecules that have an unpaired electron in their outer shell. Free radicals have been implicated to cause several diseases like liver cirrhosis, Arthritis, Diabetes, Atherosclerosis, Cancer, Ageing, Alzheimer's disease, Parkinson's disease, etc. and the compounds which can scavenge these free radicals have greater potential in ameliorating these disease processes. The human body has an inherent mechanism to reduce the free radical induced injury by endogenous enzymes superoxide dismutase, glutathione peroxidase, catalase and other substances such as Vitamin E and Ascorbic acid administered exogenously.[2] It is useful in opthalmia, hemorrhoids, dental caries, bleeding gums, ulcered oral cavity. Its paste with water is found to be anti-inflammatory, analgesic and having purifying and healing capacity for wounds. Its decoction is used as gargle in oral ulcers, sore throat. Its powder is a good astringent dentifrice in loose gums, bleeding and ulceration in gums. It is good to increase appetite, digestive aid, liver stimulant, stomachic, gastrointestinal prokinetic agent, and mild laxative, anti oxidant, ant mutageni, anti cancerous, hepato protective, cardio protective[3] anti cholinergic activiy[4] diabetes, nervine disorder & epilepsy.[5,6]

EXPERIMENTAL SECTION

MATERIALS AND METHODS

Drying and size reduction: Bark part of the two plants were carefully shade dried for 15 days. To ensure complete dryness they were kept in hot air oven at 45˚C for 5 minutes. Then they are subjected to size reduction to make powder by using mechanical grinder. The crushed mass of bark was then carried out for the process of extraction.

Extraction procedure

1. 800gms of the air-dried powdered plant material extracted with ethanol in soxhlet extractor.
2. Soxhalation of leaf powder with ethanol for 24hrs to obtain the product.
3. Then the dried marc is extracted with water by decoction.
4. Concentrate the extract by distilling of the solvent and then evaporating to dryness on the water-bath.

Preliminary Phytochemical Screening

Standard qualitative screening test of the extract was carried out for various plant constituents. The crude extract was screened for the presence or absence of secondary metabolites using standard procedures.

Anti microbial evolution[9,14]

Test Organisms Bacterial strains were obtained from National Chemical Laboratories (NCL), Pune and Microbial Type Culture Collection (MTCC), Chandigarh. The strains used for the present study were Staphycococcus aureus (NCIM 2079), Bacillus subtilis (NCIM 2063), Escherichia coli (NCIM 2931, Proteus vulgaris (NCIM 2027).

Procedure

The antimicrobial activity of the extract was assessed by disc diffusion method. Nutrient agar medium was prepared and sterilized by an autoclave. In an aseptic room, they were poured into a petridishes to a uniform depth of 4 mm and then allowed to solidify at room temperature. After solidification, the test organisms, Escherichia coli, Bacillus subtilis, Staphylococcus aureus, and Proteus vulgaris were spread over the media with the help of a sterile swab socked in bacterium and is used for antibacterial study. The ethanolic extract residues were dissolved in dimethyl sulfoxide (DMSO) to produce a concentration of 100, 250,500 µg/disc and used for the study. Ofloxacin 5 µg/disc was used as the standard. Then the sterile filter paper discs (6mm) having a capacity to hold 10 µl of extracts were immersed in definite concentration of plant extracts and placed over the solidified agar in such a way that there is no overlapping of the zone of inhibition. Plates were kept at room temperature for half an hour for the diffusion of the sample into the agar media. The organism inoculated petridishes were incubated at 37 °C for 24 hours. After the incubation period is over, the zone of inhibition produced by the samples and standard were measured. All tests were performed in triplicate.

Determination of anti oxidant Activity[11-13]

H_2O_2 scavenging activity

The H_2O_2 scavenging ability of the aqueous extract was determined spectrophotometrically, a solution of hydrogen peroxide (2 mM) was prepared in 0.17 M phosphate buffer (pH 7.4). Various concentrations of the samples (in water) were added to the reaction mixture containing 2 mM hydrogen peroxide. After 10 min incubation at room temperature, the absorbance was read against a blank at 230 nm.

H_2O_2 scavenging activity (%) = [(A control – A sample)/A blank] x 100.

Where A control is the absorbance of the control reaction (containing all reagents except the test compound), and A sample is the absorbance of the test compound.

RESULTS AND DISCUSSION

Table 1: Results of Preliminary Phytochemical Screening of extract.

S.No	Name of the test	Result
1	Amino glycosides	++
2	Phenolic compounds	++
3	Pentacyclic tri Terpenes	++
4	Tri terpinoid glycosides	++
5	Alkaloids	++
6	Flavonids	++
7	Terpenoids	++

Table 2: Anti Microbial Evolution of Compounds.

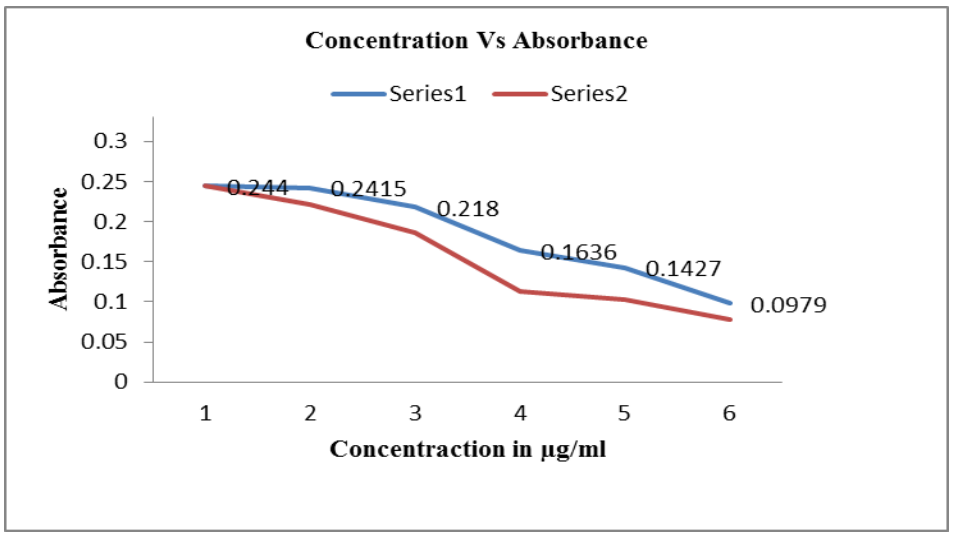
Concentration (µg/ml))	Ascorbic acid (Abs)	Aqueous extract of Swertia chirata (Abs)
5	0.2380	0.244
10	0.1719	0.2215
15	0.0469	0.1854
20	0.0415	0.1136
25	0.0410	0.1027
30	0.0390	0.0779
Control	0.2444	

Table 3: Anti oxidant activity of aqueous extract of *Swertia chirata*.

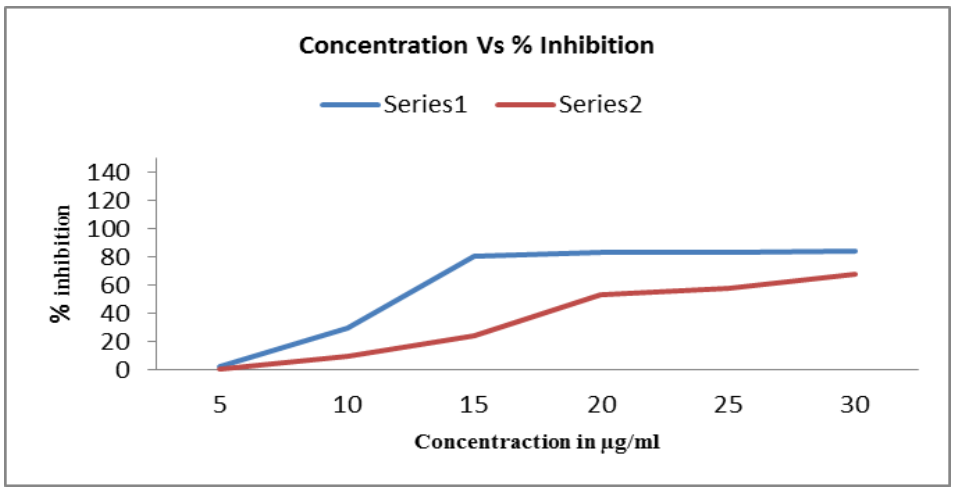
	Aqueous extract of *Swertia chirata*				Ofloxacin		
	Zone of inhibition in mm						
		100µg/ml	250mg/ml	500µg/ml	100µg/ml	250mg/ml	500µg/ml
Name of the organisms	*Staphylococcus aureus*	11	15	19	12	20	26
	Bacillus subtilis	10	14	21	14	18	24
	Escherichia coli	8	17	23	12	16	25
	Proteus vulgaris	10	15	22	12	14	22
Control	DMSO	-	-	-	-	-	-

Table 4: % inhibition of aqueous extract of *Swertia chirata* with ascorbic acid.

Concentration (µg/ml))	Ascorbic acid (% Inhibition)	Aqueous extract of Swertia chirata (% Inhibition)
5	2.618658	0.16367
10	29.66448	9.36989
15	80.81015	24.1408
20	83.01964	53.5188
25	83.22422	57.9787
30	84.04255	68.126



Graph-1: Concentration Vs Absorbance.



Graph-2: concentrations Vs % Inhibition.

DISCUSSION

The present results reveals that the aqueous extract shows the activity less than the standard. The aqueous extract of *Swertia chirata tested* for anti bacterial activity and antioxidant activity by disc diffusion method using DPPH reagent method. Here the results were

compared with the standard Ascorbic acid. The result reveals that the extract shows results less than the standard. The concentration of the extract was taken in to 5-30 µg/ml. The % of inhibition shows that the up to 30µg/ml, shows more activity than compare with other concentrations.

CONCLUSION

The outcomes of the present study indicated that the aqueous extract of the *Swertia chirata* shows the significance anti bacterial, anti oxidant activity at a concentration of 500 µg/ml, 30 µg/ml respectively. The results were compared with standards like ofloxacin, ascorbic acid respectively.

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