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IN VITRO ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF ETHANOLIC EXTRACT OF LEAVES OF *BRASSICA OLERACEA*

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ABSTRACT
The objectives of this study are to screen the antioxidant and antimicrobial capacity of the *Brassica oleracea* leaves extract. The hydro alcoholic extract of leaves of *Brassica oleracea* was studied for antioxidant activity on different *in vitro* models namely 1, 1-diphenyl, 2-picryl hydrazyl (DPPH) assay. The extract showed dose dependent free radical scavenging property in the tested models. *Brassica oleracea* leaves extract showed IC_{50} value 77.09µg/ml for DPPH method, which was comparable to that of ascorbic acid (IC_{50}=17.68µg/ml). The drug used in standard preparation was Ofloxacin, Ciprofloxacin and Clindamycin of IP grade. The antibacterial activity was performed by using 24hr culture of *Streptococcus mutans, Escherichia coli* and *Propionibacterium acnes.* In antibiogram trials, three concentrations of isolated phytochemicals were used: 25, 50, and 100 mg/ml. The placement of wells with antibiotics on the surfaces of agar immediately after inoculation with the organism studied is a key function. The antioxidant and antimicrobial effect of *Brassica oleracea* is defined in this report, and it will be used for medicinal purposes in the future.

Keywords: *Brassica oleracea*, Antioxidant activity, Antimicrobial activity.

INTRODUCTION

Exogenous chemicals and endogenous metabolic pathways in the human body produce free radicals, or highly reactive oxygen molecules. These are capable of oxidizing biomolecules such as nucleic acids, proteins, lipids, and DNA, and can cause neurological disorders, cancer, emphysema, cirrhosis, atherosclerosis, arthritis, and other degenerative diseases[1,2]. Antioxidants are molecules that stop free radicals from attacking and therefore reduce the risk of these diseases[3]. Almost all species are shielded from free radical destruction to some degree by antioxidant compounds such as ascorbic acid, tocopherol,

phenolic acids, polyphenols, flavonoids, and glutathione, as well as enzymes such as superoxide dismutase and catalase. Prior and Cao[4], reported that antioxidant supplements or dietary antioxidants protect against the damaging effects of free radicals. Natural antioxidants are currently receiving a lot of attention as a way to protect the human body, particularly brain tissues, from oxidative damage caused by free radicals. Several medicinal plants have demonstrated such efficacy by conventional psychoneuropharmacology approaches in the last two decades[5]. Brassica plants are noted for their rich bioactive structure, which is illustrated by glucosinolates, which are enzymatically cleaved to isothiocyanates by the action of myrosinase, giving these plants pronounced chemopreventive function[6]. With this in sight, the current research was designed to compare the antioxidant and antimicrobial activity of *Brassica oleracea*, which has been historically used for a variety of purposes.

MATERIALS AND METHOD

Plant material

The leaves of *Brassica oleracea* were collected from Shubham nursery, Bhopal (M.P.) in the month of Feb, 2019. The plant material used in the sample was thoroughly washed under flowing tap water and then rinsed in purified water before being allowed to dry at room temperature. The plant material was then shade dried for 3 to 4 weeks without being infected. An automated grinder was used to process dried plant material. Color, odor, flavor, and texture of powdered plant material were evaluated. For phytochemical and biological tests, dried plant material was sealed in an airtight container and stored.

Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals and solvent used in this study were of analytical grade.

Defatting of plant material

Brassica oleracea leaves were dried in the shade at room temperature. The shade-dried plant material was coarsely powdered and macerated in petroleum ether for extraction. The extraction process was proceeded until the substance had been defatted.

Extraction by Soxhletion Method[7]

56.8 gram of powdered leaves of *Brassica oleracea* were exhaustively extracted with different solvent (Chloroform, Ethyl acetate, Ethanol and Aqueous) by soxhletion method. The extract was evaporated above their boiling points. Finally, the percentage yields were calculated of the dried extracts.

In-vitro antioxidant activity of ethanolic extract of *Brassica oleracea* using DPPH method

DPPH scavenging activity was measured by the spectrophotometer (Olufunmiso *et al.,* 2011). Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentration (10- 100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly. Finally the mean was taken. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm[8].

$$Calculation\ of\ \%\ Reduction \frac{\text{Control Absorbance } - \text{Test absorbance}}{\text{Control Absorbance}} \text{X } 100$$

Antimicrobial activity of *Brassica oleracea* ethanolic extract

Pathogenic microbes used

The pathogenic bacteria and fungus used in the current study obtained from Microbial Culture collection, National Centre forcell science, Pune, Maharashtra, India.

Method of preparation

This agar medium was dissolved in purified water and then boiled in a large conical flask. Dry ingredients are put in a flask with the necessary amount of distilled water and heated to fully dissolve the medium. The flask containing medium was cotton plugged and was placed in autoclave for sterilization at 15 lbs /inch^2 (121^{o}C) for 15 minutes. After sterilization, the media in flask was immediately poured (20 ml/ plate) into sterile Petri dishes on plane surface. The poured plates were left at room temperature to solidify and incubate at 37^{o}C overnight to check the sterility of plates. The plates were dried at 50^{o}C for 30 minutes before use. Broth cultures of the pure culture isolates of those test microorganisms which are sensitive towards the phytoextracts used in present study were prepared by transferring a loop of culture into sterile nutrient broth and incubated at 37^{o}C for 24 hours. A loop full was taken from these broths and seeded onto sterile nutrient agar plates through sterile cotton swab to develop diffused heavy lawn culture. The well diffusion method was used to determine the antimicrobial activity of ethanolic extract prepared from leaves of *Brassica oleracea* using standard procedure (Bauer, 1966). There were 3 concentration used which are 25, 50 and 100 mg/ml for extracted phytochemicals in antibiogram studies. Its essential feature is the placing of wells with the antibiotics on the surfaces of agar immediately after inoculation

with the organism tested. Undiluted overnight broth cultures should never be used as an inoculum. The plates were incubated at 37ºC for 24 hr. and then examined for clear zones of inhibition around the wells impregnated with particular concentration of drug[9].

RESULTS AND DISCUSSION

DPPH radical scavenging assay measured hydrogen donating nature of extracts. Under DPPH radical scavenging activity the inhibitory concentration 50% (IC_{50}) value of *Brassica oleracea* ethanolic leaves extract was found to be 77.09µg/ml as compared to that of ascorbic acid (17.68µg/ml). A dose dependent activity with respect to concentration was observed Table 1 and Figure 1.

Table No. 1: % Inhibition of ascorbic acid and extract of *Brassica oleracea* using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Ethanolic extract
1	10	44.65	28.74
2	20	48.62	33.58
3	40	65.34	39.22
4	60	69.65	43.97
5	80	77.41	50.01
6	100	84.13	57.88
	IC 50	**17.68**	**77.09**

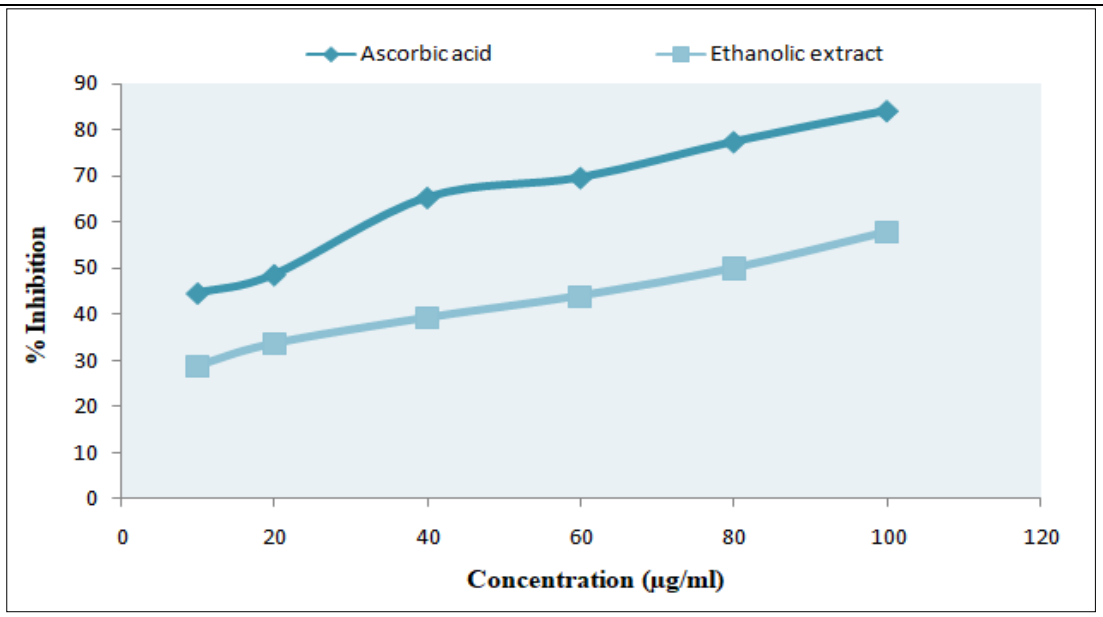

Figure 1: % Inhibition of ascorbic acid and extracts of *Brassica oleracea* using DPPH method

The well diffusion method was used to determine the antibacterial activity of the extract prepared from the *Brassica oleracea* using standard procedure of Bauer *et al*[9].The drug used in standard preparation was Ofloxacin, Ciprofloxacin and Clindamycin of IP grade. The antibacterial activity was performed by using 24hr culture of *Streptococcus mutans, Escherichia coli* and *Propionibacterium acnes.* There were 3 concentration used which are 25, 50 and 100 mg/ml for each extracted phytochemicals in antibiogram studies. It's essential feature is the placing of wells with the antibiotics on the surfaces of agar immediately after inoculation with the organism tested. Undiluted overnight broth cultures should never be used as an inoculum. The plates were incubated at 37ºC for 24 hr. and then examined for clear zones of inhibition around the wells impregnated with particular concentration of drug. The diameter of zone of inhibition of each wall was recorded table 2 and 3.

Table 2: Antimicrobial activity of *standard drug* against selected microbes

S. No.	Name of drug	Microbes	Zone of Inhibition		
			30 µg/ml	20 µg/ml	10 µg/ml
1.	Ofloxacin	*S. Mutans*	17±0.19	15±0.13	12±0.15
2.	Ciprofloxacin	*Escherichia coli*	28±0.5	21±0.57	16±0.86
3.	Clindamycin	*Propionibacterium acne*	22±0.5	18±0.57	13±0.86

Table 3: Antimicrobial activity of ethanolic extract of leaves of *Brassica oleracea* against selected microbes

S. No.	Name of microbes	Zone of inhibition Ethanolic extract		
		100mg/ml	50 mg/ml	25mg/ml
1.	*Streptococcus Mutans*	13±0.47	12±0.57	9±0.47
2.	*Escherichia coli*	15±0.47	14±0.47	11±0.47
3.	*Propionibacterium acne*	16±0.5	12±0.47	10±0.47

CONCLUSION

In this study, we analyzed the antioxidant and antimicrobial activities of *Brassica oleracea*. The overall results of the present study suggest that the Ethanolic leaf extract of *Brassica oleracea* could be useful as a source of natural antioxidant agents. In addition, the leaf extract of *Brassica oleracea* was shown to

possess notable pharmaceutical activities, indicating that *Brassica oleracea* should be considered as a useful source for herbal medicine. The variation in pharmaceutical activities between organic extracts indicates that the comparative analysis of the metabolome in leaf extracts will be required for the isolation and characterization of the active compounds in *Brassica oleracea*.

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