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An Experimental Study On In Vitro Antioxidant And Antimicrobial Activity Of Ethanolic Extract Of Brassica Oleracea Leaves

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ABSTRACT
Green cabbage, or Brassica oleracae, is a herbaceous biennial plant whose leaves assemble into a compact head. It is an edible vegetable that has long been used as a medicinal herb, with several health advantages claimed. The purpose of this study is to evaluate the extract of Brassica oleracea leaves for antioxidant and antibacterial properties. Brassica oleracea leaf hydroalcoholic extract was tested for antioxidant activity using a variety of in vitro models, including the 1, 1-diphenyl, 2-picryl hydrazyl (DPPH) assay. In the investigated models, the extract exhibited dose-dependent properties related to free radical scavenging. The DPPH technique yielded an IC50 value of 78.19 µg/ml for the extract of Brassica oleracea leaves, which was similar to ascorbic acid (IC50=18.78 µg/ml). The medication used in the usual formulation was IP-grade ofloxacin and ofloxacin. Escherichia coli and Streptococcus mutans were cultured for 24 hours in order to test the antibacterial activity. Three concentrations of extracted phytochemicals—25, 50, and 100 mg/ml—were employed in antibiogram experiments. One important purpose is to promptly insert wells containing antibiotics on the agar surfaces following the organism under study's inoculation. This research defines Brassica oleracea's antioxidant and antibacterial properties, which will be used to medicine in the future.

KEY-WORDS: *Brassica oleracae,* Ofloxacin, Antioxidant, Antimicrobial, In-vitro

INTRODUCTION

Free radicals, or very reactive oxygen molecules, are produced by the human body through both endogenous metabolic processes and exogenous substances. These can induce neurological illnesses, cancer, emphysema, cirrhosis, atherosclerosis, arthritis, and other degenerative diseases. They can also oxidize biomolecules such nucleic acids, proteins, lipids, and DNA.[1] Antioxidants are chemicals that lower the risk of some diseases by preventing free radicals from attacking. Antioxidant substances including ascorbic acid, tocopherol, phenolic acids, polyphenols, flavonoids, and glutathione, as well as enzymes like superoxide dismutase and catalase, protect almost all species against the damaging effects of free radicals to some extent.[2]According to research, dietary or supplemental antioxidants guard against the harmful effects of free radicals. The utilization of natural antioxidants to shield human tissues—especially those of the brain—from oxidative damage brought on by free radicals is gaining a lot of interest these days.[3] In the past 20 years, a number of medicinal plants have proven to be effective when tested using traditional psychoneuropharmacology methods.[4]
Approximately 80% of the rural population receives their main healthcare from herbal medicine, demonstrating the effectiveness of medicinal plants as a source of both traditional and contemporary medications.[5] Many doctors recommend herbal medications due to its efficacy, low cost, and lack of negative effects. Consequently, the study of plants for their pharmacological properties has gained significance.[6]
Because of its accessibility in local markets and growing popularity among consumers, Brassica oleracae, a leafy garden plant, is one of the most significant vegetables consumed globally.[7] It has a lot of phytochemicals, including glucosinolates and flavonoids. It is an excellent source of chemicals that promote health and have been shown to have preventative benefits against diabetes mellitus, cancer, atherosclerosis, and nephritis. In a similar vein, not many research have emphasized the plant's significance as a possible source of antifungal agent.[8]
It has been found that the breakdown products of glucosesinolates in cabbage have antibacterial properties. New bacterial strains that are Multi Drug Resistant (MDR), or almost resistant to the majority of commonly used antibacterial medications, have emerged as a result of an increase in resistant strains of clinically significant

pathogens. A higher risk of morbidity and death has been attributed to new antibacterials' lack of accessibility and expensive cost.[9] As a result, there was a surge in the hunt for more potent antibacterial medicines derived from plants to treat illnesses caused by multidrug resistant bacteria. Therefore, the purpose of the study was to assess the leaf extract of Brassica oleracae's antioxidant and antibacterial properties against clinical isolates of pathogenic bacteria that are multidrug resistant.[10]

MATERIAL AND METHOD

Plant material

The Brassica oleracea leaves were gathered from Jharkhand's Uttam Garden & Nursery. Before being allowed to dry at room temperature, the plant material used in the sample was carefully cleaned under running tap water and then rinsed in purified water. After three to four weeks of shade drying, the plant material was free of infections. Dried plant material was processed using an automatic grinder. The dried plant material's color, flavor, odor, and texture were assessed. Dried plant material was maintained in an airtight container after being sealed for phytochemical and biological testing.

Chemical reagents

All the chemicals and solvent used in this study were of analytical grade

Extraction by Soxhletion Method

By using the soxhletion technique, 54.6 grams of powdered Brassica oleracea leaves were thoroughly extracted using a variety of solvents, including ethanol, chloroform, ethyl acetate, and aqueous. Above the points at which they boiled, the extract evaporated. Lastly, the dried extracts' percentage yields were determined.

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

The spectrophotometer was used to test the DPPH scavenging activity. An initial absorbance was obtained by preparing a stock solution (6 mg in 100 ml methanol) and using 1.5 ml of it in 1.5 ml of methanol. After 15 minutes, a decrease in absorbance was seen in the presence of sample extract at various concentrations (10–100 µg/ml). After taking 1.5 ml of the DPPH solution and adding methanol to make it up to 3 ml, the absorbance was measured right away at 514 nm for the control reading. A series of volumetric flasks were filled with 1.5 ml of DPPH and 1.5 ml of the test sample at various concentrations. The final volume was adjusted to 3 ml using methanol. Three test specimens were obtained and subjected to identical processing. And lastly, the taken mean. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 514 nm.

Antimicrobial activity of *Brassica oleracea* ethanolic extract Pathogenic microbes used

The pathogenic bacteria and fungus used in the current study obtained from Jharkhand, India.

Method of preparation

In a large conical flask, the agar medium was dissolved in filtered water and then brought to a boil. To completely dissolve the medium, dry materials are placed in a flask with the required volume of distilled water and heated. After plugging the flask with the medium with cotton, it was autoclaved for 15 minutes at 15 pounds per inch, or 121 degrees Celsius. Following sterilization, sterile Petri dishes with a flat surface were filled with the medium in the flask (20 ml/plate). The poured plates were allowed to harden at room temperature before being incubated at 37°C for the whole night to ensure plate sterility.Prior to usage, the plates were dried at 50°C for 30 minutes. By transferring a loop of culture into sterile nutritional broth and incubating it at 37oC for 24 hours, broth cultures of the pure culture isolates of those test microorganisms that are sensitive to the phytoextracts utilized in this investigation were created. To create diffused heavy lawn culture, a loop full of these broths was extracted and seeded onto sterile nutrient agar plates using a sterile cotton swab. The antibacterial activity of an ethanolic extract made from Brassica oleracea leaves was assessed using the well diffusion technique in accordance with established protocol.

In antibiogram investigations, three concentrations of extracted phytochemicals—25, 50, and 100 mg/ml—were employed. The placement of wells containing antibiotics on agar surfaces as soon as the organism under test is inoculated is a crucial component of this technique. Never use undiluted overnight broth cultures as an inoculum. Following a 24-hour incubation period at 37°C, the plates were checked for distinct zones of inhibition surrounding the wells that had been impregnated with a specific drug concentration.

RESULTS AND DISCUSSION

The extracts' ability to donate hydrogen was assessed using the DPPH radical scavenging test. The extract of ethanolic leaves from Brassica oleracea was shown to have an inhibitory concentration 50% (IC50) value of

78.19 µg/ml under DPPH radical scavenging activity, which was higher than that of ascorbic acid (18.78 µg/ml). Table 1 and Figure 1 show a dose-dependent action with regard to concentration. The antibacterial activity of the Brassica oleracea extract was assessed using the well diffusion technique in accordance with Bauer et al9. standard protocol.The medication used in the usual formulation was IP-grade ofloxacin and ofloxacin.

The activity of the antibacterial agent was carried out utilizing a 24-hour culture of Escherichia coli and Streptococcus mutans. In antibiogram tests, three concentrations of isolated phytochemicals—25, 50, and 100 mg/ml—were employed. Its crucial component is the instantaneous placement of wells containing antibiotics on agar surfaces following the organism under test's inoculation. It is never appropriate to utilize undiluted overnight broth cultures as an inoculum. Following a 24-hour incubation period at 37°C, the plates were checked for distinct zones of inhibition surrounding the wells that had been impregnated with a specific drug concentration. Tables 2 and 3 show the diameter of each wall's zone of inhibition.

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	45.75	29.84
2.	20	49.72	34.68
3.	40	66.35	40.32
4.	60	70.75	44.98
5.	80	78.51	51.02
6.	100	8.23	58.98
IC 50		**18.78**	**78.19**

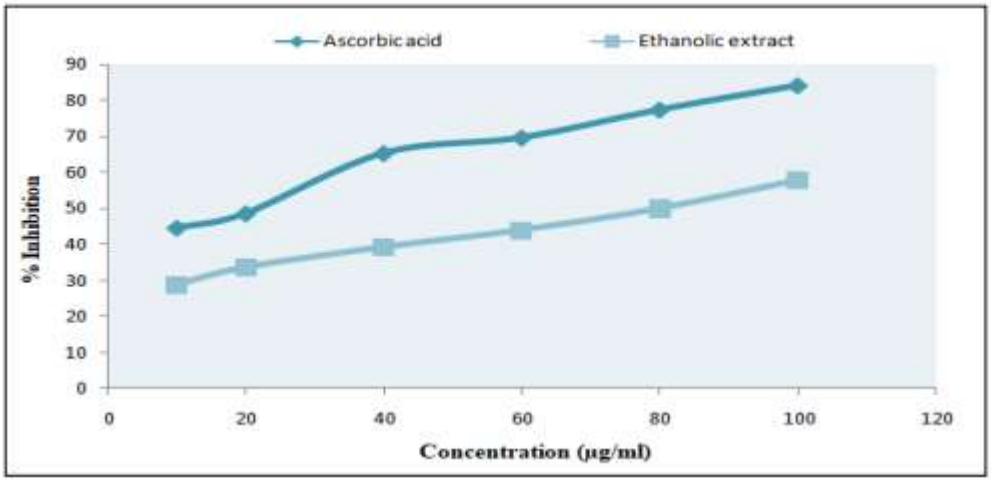

Figure 1: % Inhibition of ascorbic acid and extracts of *Brassica oleracea* using DPPH method

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

.N.	Drug	Microbes	Zone of Inhibition		
			30 µg/ml	20 µg/ml	10 µg/ml
1.	Ofloxacin	*Streptococcus Mutans*	16±0.19	14±0.03	11±0.05
2.	Ciprofloxacin	*Escherichia coli*	27±0.5	20±0.47	15±0.76

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

S.N.	Name of microbes	Zone of inhibition		
		Ethanolic extract		
		100mg/ml	50 mg/ml	25mg/ml
1.	*Streptococcus Mutans*	12±0.37	11±0.47	8±0.37
2.	*Escherichia coli*	14±0.37	13±0.37	10±0.37

CONCLUSION

In this investigation, we examined Brassica oleracea's antibacterial and antioxidant properties. Overall, the study's findings point to the potential use of Brassica oleracea leaf extract as a natural source of antioxidants.

Furthermore, it has been demonstrated that Brassica oleracea leaf extract possesses noteworthy pharmacological activity, suggesting that Brassica oleracea might be a valuable source for herbal medicine. The different pharmacological effects of the organic extracts suggest that in order to isolate and characterize the active chemicals in Brassica oleracea, a comparative study of the metabolome in leaf extracts would be necessary.

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