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Research Article

IN VITRO ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF *BRASSICA OLERACEA*

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ABSTRACT

Objective: To investigate the antimicrobial activity and phytochemical analysis of *Brassica oleracea.*

Methods: The antimicrobial activity was evaluated using agar well diffusion and micro dilution methods against the bacterial (*Pseudomonas aeruginosa, Shigella flexneri, E.coli* and *Klebsiella pneumoniae*) and fungal (*Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus* and *Cladosporium* species) isolates. The extraction of the vegetable was carried using the solvents namely aqueous, acetone, petroleum ether and chloroform. Phytochemical and FT-IR analysis was carried out in the acetone extract.

Results: Among the four extracts tested, acetone extract exhibited maximum activity against *Pseudomonas aeruginosa, Shigella flexneri, E.coli* and *Klebsiella pneumoniae* and a narrow range of activity was exhibited against aqueous, petroleum ether and chloroform extracts. *Aspergillus flavus* and *Aspergillus niger* was found to be sensitive to acetone extracts when compared with *Aspergillus fumigatus* and *Cladosporium* species. Phytochemical analysis of *Brassica oleracea* extracts showed the presence of secondary metabolites like phenolics, alkaloids, flavonoids and saponins. The FT-IR analysis has revealed the presence phenols, alcohol, amines and carboxylic acid as functional groups in *Brassica oleracea*.

Conclusion: From this study, it can be concluded that *Brassica oleracea* exhibits antimicrobial activity against certain microorganisms.

Keywords: Antibacterial, Antifungal, *Brassica oleracea,* Phytochemical analysis, FT-IR analysis

INTRODUCTION

Infectious diseases continue to be a serious burden around the world, in developing and industrialized countries alike [1]. The history of prevention and treatment of disease or the science of healing started from ancient period, when it was considered more as an art than a science. A remarkable scientific breakthrough was developed in the 19th & 20th centuries [2]. Now-a-day's multidrug resistance from indiscriminate usage of the antibiotics has led to pursuance of natural drugs. Herbal medicines are becoming popular in modern world as people resort to natural therapies. Natural products isolated from higher plants have been providing novel clinically active drugs which created the scientist to identify the potent and effective antimicrobial agents of plant origin to replace the antibiotics [3]. It is evident that plants produce a diverse range of bioactive molecules which can inhibit the growth of microbes.

Vegetables have been analysed as potent medicine and man is able to obtain from them a wondrous assortment of industrial chemicals. In recent years population continues to explode and microbial disaster may occur. So vegetables with possible antimicrobial activity should be tested against an appropriate microbial model to confirm its activity and to ascertain the parameter associated with it.

Cruciferous vegetables are one of the dominant food crops which have high vitamin C, soluble fibre and contain multiple nutrients and phytochemicals with potential anticancer properties. *Brassica oleracea* (Cauliflower*)* belongs to the family *Brassicaceae* is an annual plant that reproduces by seed. Cauliflower is low in fat, but high in dietary fibre, potassium, folate, water and vitamin and possesses a high nutritional density. Cauliflower contains several phytochemicals which are beneficial to human health. It contains sulforaphane which protect against cancer, glucosinolates, carotenoids, indole-3-carbinol, isothiocyanates, dithiolethiones and phenols that enhances DNA repair and acts as an estrogen antagonist, slowing the growth of cancer cells. A high intake of cauliflower has been associated with reduced risk of aggressive prostate cancer. The leaf juice of cauliflower was found to possess antibacterial activity [4]. Since the literature regarding the present work is meager an attempt has been made to evaluate the antimicrobial activity and phytochemical analysis of *Brassica oleracea* extracts against the pathogenic microbes.

MATERIALS AND METHODS

Vegetable collection

Brassica oleracea was collected from the local markets of Coimbatore, Tamil Nadu, India on September 2011. The vegetable was washed thoroughly under running tap water to remove dirt and then shade dried at room temperature for a week. They were ground into fine particles after drying and kept in closed container before being stored at room temperature until further used. The date, place and information of vegetable collection were recorded. The voucher of the specimen and the taxonomy of the vegetable were deposited in the Department of Botany, Avinashilingam Institute for Home Science and Higher education for Women, Coimbatore, Tamil Nadu, India.

Extraction and sample preparation

Ten grams of the ground sample of *Brassica oleracea* was weighed and homogenized with 100ml of petroleum ether, acetone and chloroform separately. The crude preparation was left overnight in the shaker at room temperature and then centrifuged at 4000 rpm for 20 minutes. The supernatant containing the vegetable extract was then transferred to a pre-weighed beaker and the extract was concentrated by evaporating the solvent at 60ºC. For the preparation of aqueous extract, 10g of the sample was added with 100ml of distilled water and kept in a shaker at 90-120 rpm for 24h at 30ºC. The mixture was boiled at 60ºC for 3h and concentrated to one fourth of the original volume. The extracts were then concentrated to dryness under vacuum and reduced pressure using rotary evaporator to obtain the concentrated extracts. Then the crude extracts were dissolved in known volume of dimethylsulphoxide (DMSO) to obtain a final concentration of 20mg/ 5 µl. The aliquot was stored until it was used.

Microbial strains

Four bacterial strains (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Shigella flexneri)* and fungal strains (*Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus* and *Cladosporium* species) were used in the present study were the clinical isolated obtained from P.S.G. Hospitals, Coimbatore, Tamil Nadu, India.

Antimicrobial assay

Well diffusion method

The agar well diffusion method was employed for the determination of antimicrobial activity of the extracts [5]. To brief, wells were made in Muller Hinton agar plates (Himedia, Mumbai, India) and Rose Bengal Chloramphenical agar (Himedia, Mumbai, India) plates using cork borer (5mm diameter) and the inoculum containing 50 µl of bacteria and fungi were swabbed on the above plates with a sterile swabs separately. 20 µl of the *Brassica oleracea* extracts, control (DMSO) and standard antibiotics (4mg of Chloramphenical and nystatin) (Himedia, Mumbai, India) was filled in wells with the help of micropipette separately. The plates were then incubated at 37º C for 24 hours for bacteria and at room temperature (25 -30º C) for five days for fungal strains. The samples were tested in duplicates and the diameter for the zone of inhibition was measured as millimeter (mm).

Microdilution method

The minimum inhibitory concentration (MIC) was determined by micro dilution method using serially diluted *Brassica oleraceae* extracts**.** The acetone extract was diluted to different concentrations in sterile Muller Hinton broth using 96 - well plates. The microorganism suspension of 50µl was added to the broth dilutions and was incubated at 37ºC for 24 hours. The MIC values were taken as the lowest concentration of the extract in the well of the microtiter plate that showed no turbidity after incubation. The turbidity of the wells in the microtiter plate was interpreted as visible growth of microorganisms [6].

Phytochemical analysis

The extracts obtained from *Brassica oleraceae* were qualitatively tested to identify the presence of phytochemicals [7].

FT – IR analysis

FT-IR (Fourier Transform Infrared) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups). The wavelength of light absorbed is characteristic of the chemical bond which can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. For the FT-IR study dried powder of acetone extract (10 mg) of *Brassica oleraceae* was taken in a mortar and pestle and ground with 2.5 mg of dry potassium bromide (KBr). The powder so obtained was filled in a 2 mm internal diameter micro-cup and loaded onto FT- IR set at 26°C ± 1°C. The samples were scanned using infrared in the range of 3500–500 cm^{-1} using Fourier Transform Infrared Spectrometer (Shimadzu, IR Affinity 1, Japan). The spectral data obtained were compared with the reference chart to identify the functional groups present in the sample [8].

RESULTS

Antibacterial activity of *Brassica oleracea*

The results of agar well diffusion method of *Brassica oleracea* using different solvents were depicted in Table 1. Among the four different extracts acetone extract proved to exhibit maximum antimicrobial activity against the tested bacterial and fungal isolates. All the bacterial and fungal isolates were moderately susceptible to chloroform and aqueous extracts and appeared to be more resistant to petroleum ether extract (Table 1).

Table 1: Antimicrobial activity of different extracts of *Brassica oleracea* using agar well diffusion method

Microorganisms	Zone of inhibition in diameter (mm)					
	Chloroform	Acetone	Aqueous	Petroleum ether	Positive control	Negative control
Escherichia coli	15	28	14	9.5	16	0
Pseudomonas aeruginosa	19	29	17	12	19	0
Klebsiella pneumoniae	21	27	19	17.5	21	0
Shigella flexneri	23	22.5	15	13	14	0
Aspergillus fumigatus	21	22	16.5	14	12	0
Cladosporium species	18.5	20	15	11.5	15	0
Aspergillus flavus	17	28	16	12	21	0
Aspergillus niger	20	24.5	17	13	19	0

Positive control – Chloramphenicol, Nystatin, Negative control - DMSO

The mean inhibition zone for the tested bacterial and fungal isolates ranged from 9 – 29mm and 11 -28mm respectively, indicating a remarkable antimicrobial effect when compared with that of chloramphenicol and nystatin, the positive control which ranged from 14 – 21mm and 12 -21 mm respectively.

The minimum inhibitory concentration (MIC) of the extracts to inhibit the microorganisms was determined using the microdilution method. Since the acetone extract showed the maximum zone of inhibition, the MIC was determined only with this extract. Table 2 depicts the MIC values of the extract against the tested bacterial and fungal isolates.

Table 2: Minimum Inhibitory Concentration (MIC) of *Brassica oleraceae* against the tested microorganisms

Microorganisms	Acetone extract (mg/µl)					
	40	20	10	5	2	1.25
Escherichia coli	0.52	0.42	0.28	0.20	0.17	0.15
Pseudomonas aeruginosa	0.29	0.21	0.17	0.19	0.14	0.09
Klebsiella pneumoniae	0.31	0.27	0.21	0.18	0.11	0.10
Shigella flexneri	0.47	0.42	0.20	0.17	0.14	0.10
Aspergillus fumigatus	0.54	0.48	0.51	0.37	0.31	0.25
Cladosporium species	0.49	0.41	0.37	0.30	0.24	0.09
Aspergillus flavus	0.34	0.29	0.21	0.11	0.09	0.02
Aspergillus niger	0.62	0.56	0.49	0.33	0.22	0.14

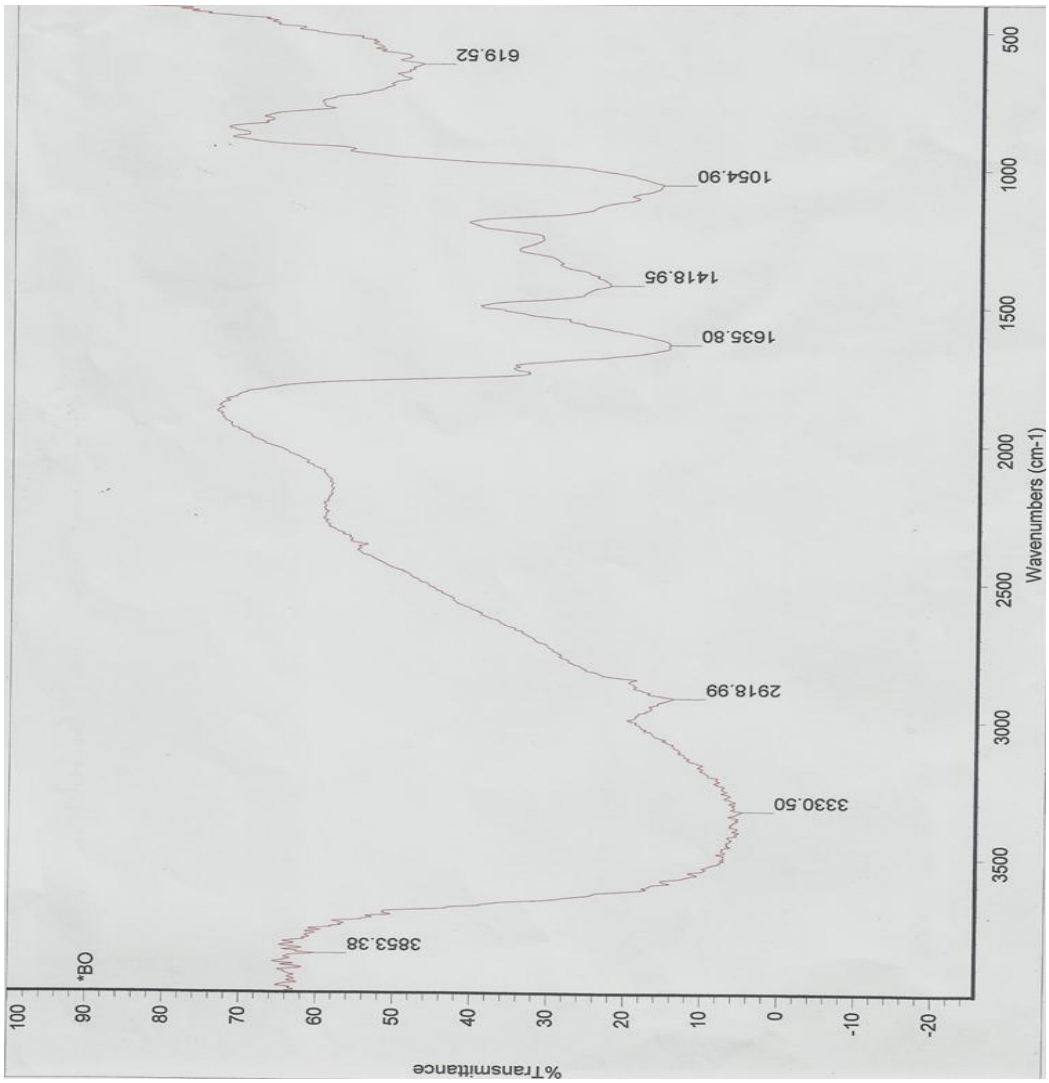
The logarithmic OD values were demonstrated for each tested bacterial and fungal isolates and the OD values decreased in a dose dependent manner. The results showed that the minimum concentration of 12.5 mg/ml extract could resist the growth of tested bacterial and fungal isolates. The results further validate the activity of acetone extracts against all the tested bacterial and fungal isolates.

The results obtained in the qualitative phytochemical analysis of various extracts of *Brassica oleracea* indicated the presence of alkaloids, phenols and saponins in all the extracts. Flavonoids are found to be present in aqueous and petroleum ether extracts. It was observed that amino acid, tannins and quinones were absent in all the extracts of *Brassica oleracea* (Table 3).

Table 3: Qualitative phytochemical analysis of *Brassica oleracea*

Phytochemicals	*Brassica oleracea* extracts			
	Acetone	Aqueous	Chloroform	Petroleum ether
ALKALOIDS				
Mayer's Reagent	+	+	+	+
Dragendroff's Reagent	+	+	+	+
Wagner's Reagent	+	+	+	+
Hager's test	+	+	+	+
PHENOLS				
Ferric chloride test	+	+	+	+
Lead acetate test	+	+	+	+
AMINO ACID				
Ninhydrin test	-	-	-	-
FLAVONOIDS				
Schinoda's test	-	+	-	+
Ammonia Test	+	+	+	+
SAPONINS				
Froth test	+	+	+	+
Sodium Bicarbonate test	+	+	+	+
TANNINS				
Breamer's test	-	-	-	-
QUINONES				
Borntrager's test	-	-	-	-

The FT –IR spectrum of *Brassica oleracea* in the range of 500 – 3500 cm^{-1} revealed the presence of many functional groups. It exhibits the peak at 3330, 1635, 1418 and 1054 cm^{-1} which indicates the presence of –OH, -COOH, -NH and C=O groups respectively (Figure 1).



**** BO- Brassica oleracea***

Fig. 1: FT-IR Spectral analysis of *Brassica oleracea*

DISCUSSION

In recent years the use of plants as source of drugs has been increased for the treatment of infectious diseases. Hence, there is a need to move towards the traditional medicine which can serve as novel therapeutics. Numerous studies have highlighted the potential importance of vegetables as a source of medicine which has been inherited as an important component of the health care system in India. Vegetable extracts are given singly or as concoctions for the treatment of microbial diseases [9]. In the present study notable activity was observed against all the tested micro organisms. In an overview of the bioactivity data obtained from the current study, it can be highlighted that the tested extracts have potential to inhibit bacteria and fungi. *Pseudomonas aeruginosa* exhibited more inhibitory activity which represents the role of phytoconstituents towards the action of permeability on peptidoglycon layer. The maximum antibacterial activity exhibited by the acetone extract may create an acidic environment that caused the disruption of bacterial and fungal cell membrane [10]. The cell wall synthesis is an alternative target for antifungal agents because it is unique to the pathogen. Because of its uniqueness, fungal cell wall offers a unique target site for developing new antibiotic [11].

The phytochemical obtained from the vegetables have a potential role in health care industries and also serve as a lead chemicals for new drug development with diverse range of antimicrobial properties. Bioactive substances from this plant can therefore be employed in the formulation of antimicrobial drugs for the treatment of various bacterial and fungal infections. Phytochemical agents act as antimicrobial agent by inhibiting the extracellular enzyme acting on the substrates required for microbial growth or by inhibiting oxidative phosphorylation of microbial metabolism [12].The phytochemical contents of the leafy vegetables serve as supplements for food and also have the potential to improve the health status of its users as a result of the presence of various compounds vital for good health.

Hence, the recent research showed that the complex mixture of phytochemicals in vegetables provides a better protective effect on health than single phytochemicals [13]. Therefore, the complex components of cauliflower extract needs to be scrutinized in depth, in order to find out the best mixture of effective components that had role in the currently shown antibacterial and antifungal activity. The presence of characteristic functional groups may be responsible for the medicinal properties of *Brassica oleracea* which contain high therapeutic content. Determination of respective antimicrobial potential and toxicological evaluation of these extracts with the view to formulate novel chemotherapeutic agents to be used future is worth mentioning.

To conclude, the present bioprospecting study justifies the medicinal uses of the vegetables and also reveals the potentialities to isolate a promising natural compound for the management of the bacterial and fungal infectious disease.

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