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PHARMACOGNOSTICAL PROSPECTIVE AND IN-VITRO ANTI OXIDANT AND ANTICANCER ACTIVITIES OF *BRASSICA OLERACEA* VAR. *ITALICA*, BRASSICACEAE.

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ABSTRACT

The routine life style of people living in metropolitan cities is severely affected by various ailments which are caused by the unhealthy life style of the people who work with lots of stress in order to full fill their day to day assignments. Stress has been proved to be root cause of various disorders and abnormalities in the physiological functioning of the normal human body. As mentioned in ancient scriptures, our ancestors have been taking food items which have the potentials to reduce free radicals generated in our bodycaused due to stress, this phenomena is called as free radical scavenging activity. Recently antioxidants have attracted considerable attention in relation to radicals and oxidative stress, cancer prophylaxis and therapy, and longevity. Humans have been bestowed by nature which has a vast varieties of plants which are having anti oxidant activity, one such plant is *Brassica oleracea var. italica,* family Brassicaceae commonly called as Broccoli. The present research focuses on the anti oxidant and anticancer activity of various extracts of Broccoli. Nitric oxide and hydrogen peroxide scavenging method used for antioxidant activity and Microculture tetrazolium (MTT) assay is adopted for in vitro anticancer study. Based on the radical scavenging behaviour the methanolic extract of flowers of B.oleracea has been chosen for MTT assay. The results obtained from this preliminary screening are statistically significant for killing the cancer cell line invitro. This study might be useful in establishing the medicinal claims of the selected plant and in the scientific standardization of the raw materials.

Key Words: *Brassica oleracea var. italica,* Brassicaceae, methanolic extract, free radical scavenging, MTT assay.

INTRODUCTION

Antioxidants are compounds capable to either delay or inhibit the oxidation processes which occur under the influence of atmospheric oxygen or reactive oxygen species. Antioxidants are involved in the defence mechanism of the organism against the pathologies associated to the attack of free radicals [1]. Endogenous antioxidants are enzymes, like superoxide dismutase, catalase, glutathione peroxidase or non-enzymatic compounds, such as uric acid, bilirubin, albumin, metallothioneins [2]. When endogenous factors cannot ensure a rigorous control and a complete protection of the organism against the reactive oxygen species, the need for exogenous antioxidants arises, as nutritional supplements or pharmaceutical products, which contain as active principle an antioxidant compound [3]. Amongst the most important exogenous antioxidants, vitamin E, vitamin C, β-carotene, vitamin E, flavonoids, mineral Se are well known, but also vitamin D and vitamin K3 [4]. Recently, antioxidants have attracted considerable attention in relation to radicals and oxidative stress, cancer prophylaxis and therapy, and longevity [5]. Phenols and polyphenols are the target analytes in many such cases; they may be detected by enzymes like tyrosinase or other phenol oxidases, or even by plant tissues containing these enzymes [6]. The recommendations based on epidemiological studies are such that fruits, vegetables and less processed staple foods ensure the best protection against the development of diseases caused by oxidative stress, such as cancer, coronary heart disease, obesity, type 2 diabetes, hypertension and cataract [7,8].

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Broccoli (*Brassica oleracea L. italica*) has been marketed as a health-promoting food because it naturally has high content of bioactive phytochemicals such as glucosinolates, phenolic compounds, vitamin C and mineral nutrients [9-11]. Thus, a diet rich in broccoli plays a role in the prevention of chronic diseases, such as cardiovascular and carcinogenic pathologiesand breast and prostate cancers. Broccoli has also been found to exhibit antioxidant activity that prevents oxidative stress related to many diseases [12-15].

MATERIALS AND METHODS

Pharmacognostical Prospective of *B.oleracea*

The systematic Pharmacognostical study of *B.oleraceae* was done to authenticate the plant. The sample was collected from the local super market and it was taxonomically authenticated by Dr.C.Ramesh, botanist from French institute of Puducherry. The morphological and microscopical examinations have been performed using reference book. The ash value, extractive value and preliminary phytochemical screenings were done using standard protocol to assess its quality and efficacy [16,17].

In vitro antioxidant activity by Nitric Oxide & Hydrogen peroxide method

Nitric oxide scavenging activity

The nitric oxide scavenging activity of Broccoli was determined according to the method (Green *et al*., 1982) [18]. Aqueous solution of sodium nitroprusside spontaneously generates nitric oxide (NO) at physiological pH, which interacts with oxygen to produce nitrate ions and which was measured calorimetrically. 3ml of reaction mixture containing sodium nitro prusside, 10mM in phosphate buffered saline (PBS) and various concentrations of the extracts was incubated at 37°C for 4 hours. Control without test compound was kept in an identical manner. After incubation, 0.5ml of Griess reagent was added. The absorbance of the chromophore formed was read at 546nm. The percentage inhibition of Nitric oxide generation was measured by comparing the absorbance value of control and those of test compounds.

Hydrogen peroxide scavenging (H_2O_2) assay:

A solution of hydrogen peroxide (40 mM) is prepared in phosphate buffer (50 mM pH 7.4). The concentration of hydrogen peroxide is determined by absorption at 230 nm using a spectrophotometer. Extract (20–60 µg/mL) in distilled water is added to hydrogen peroxide and absorbance at 230 nm is determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging is calculated as follows [19]

% scavenged (H_2O_2) = $[(A_i-A_t)/ A_i] \times 100$

Where A_i is the absorbance of the control.

A_t is the absorbance of the test.

In vitro anti cancer activity

Microculture tetrazolium (MTT) assay

The monolayer cell culture was trypsinated and the cell count was adjusted to 1.0 x 10^5cells/ml using medium containing 10% new born calf serum. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 hours, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once and 100µl of different drug concentrations was added to the cells in microtitre plate. The plate was then incubated at 37°C for 3 days in 5% CO2 atmosphere and microscopic examination was carried out and the observations recorded every 24 hours. After 72 hours, the drug solutions in the wells were discarded and 50 µl of MTT was added to each well. The plates were gently shaken and incubated for 3 hours at 37°C in 5% CO2 atmosphere. The supernatant was removed and 50 µl of propanol was added and the plates were gently shaken to solubilize the formazan. The absorbance was measured using a micro plate reader at a wavelength of 540 nm. The percentage growth inhibition and percentage cell protection was calculated using the formula [20]

$$\% \text{ growth inhibition} = 100 - \frac{\text{Mean O.D of individual test group}}{\text{Mean O.D of control group}} X100$$

RESULTS AND DISCUSSION

Table 1. Pharmacognostical studies of *B.oleracea*.

Parameters	Values obtained % w/w on dry weight basis
Ash value	7.36 ± 0.12
Water soluble ash	6.65 ± 1..02
Acid insoluble ash	1.11 ± 0.04
Sulphated ash	3.78 ± 0.12
Loss on drying	2.55 ± 0.10
Foreign organic matter	0.05 ± 0.08
Water soluble extractive	15.23 ± 1.12
Alcohol soluble extractive	18.24 ± 0.06

Values are Mean ± SEM

Table 2. Phytochemical screening of extract of *Brassica oleracea*

S.No	Constituents	Result
1.	Carbohydrates	+
2.	Proteins	+
3.	Amino acids	+
4.	Alkaloids	+
5.	Steroids	+
6.	Phenols	+
7.	Flavonoids	+
8.	Glycosides	+
9.	Saponins	-
10.	Terpenes	+
11.	Tannins	+

(+) indicates positive, (-) indicates negative

Table 3. *In-vitro* Antioxidant Activity of various extracts of *Brassica oleracea*

Method	Conc. (µg)	Standard	Hexane	Chloroform	Methanol	Water
Nitric oxide (%inhibition)	100	**49.94**	18.60	21.71	*37.75*	21.22
	200	**57.00**	23.68	31.60	*46.17*	33.21
	400	**61.80**	24.81	41.36	*50.16*	39.10
	800	**72.75**	22.68	46.25	*67.90*	43.59
	1000	**87.29**	27.40	53.80	*78.73*	53.57
H_2O_2 (%inhibition)	100	**30.07**	32.12	29.36	*20.05*	48.99
	200	**49.95**	39.23	38.78	*45.25*	45.44
	400	**59.98**	44.16	47.21	*55.89*	42.37
	800	**60.40**	46.74	50.31	*58.89*	39.56
	1000	**70.15**	47.62	55.31	*68.49*	38.51

Table 4. Percentage cytotoxicity of methanolic extract of *B.oleracea* on HeLa and MCF7 cell line by MTT assay

Concentration (µg/ml)	Mean absorbance		% Growth inhibition	
	HeLa	MCF7	HeLa	MCF7
100	0.052±0.12	0.087±0.01	79.60	49.41
75	0.068±0.10	0.109±0.40	73.33	36.62
50	0.097±0.02	0.122±0.08	61.96	29.06
25	0.118±0.08	0.140±0.02	53.72	18.60
Control	0.255±0.02	0.172±0.01	0	0

Experiments were done in triplicate n=3.

DISCUSSION

A preliminary Pharmacognostical study of *B.oleracea* reveals that the plant was botanically identified and the physico-chemical analysis of the flower part of the plant shows the values are lies in between the normal range as mentioned in WHO quality control of herbal drugs for human oral consumption. The plant part is already been used as a commercial vegetable for various nutritional benefits. The preliminary phytochemical screening shows the presence of all plant phytoconstituents except saponins. The values are very much helpful to establish Pharmacopoeial standards for the plant concerned.

The *in vitro* antioxidant activity of the various polar extracts of B.*oleracea* shows the methanolic extracts possess significant effect than the other three. Based on the results of antioxidant activity, the methanolic extract was particularly concentrate for the screening of cytotoxic effect on two cancer cell line viz HeLa (liver), MCF7 (cervical). The findings of the *in vitro* cytotoxic activity clearly demonstrates that the methanolic extract of flowers of B.*oleracea* possess prominent anticancer effect on dose dependent manner. The findings of the results synergize the previous results on the same plant and enlighten the broad usage of the Broccoli as commercial, nutritional and medicinal vegetable.

CONCLUSION

The present study involves the Pharmacognostic prospective such as collection, authentication, successive solvent extraction, proximate analysis and preliminary phytochemical screening of fresh flowers of *Brassica oleracea*, Brassicaceae. The results obtained from this *invitro* anti-oxidant and anti-cancer studies indicated that the extracts of broccoli have significantly reduces the release of free radicals by Nitric acid and hydrogen peroxide radical scavenging assay. Out of the several extracts the methanolic extracts showed maximum free radical scavenging activity than the other extracts. *In vitro* cytotoxicity of methanolic extract of Broccoli on HeLa and MCF7 cell line by MTT assay was performed and the results were indicated that the methanolic extract was significantly inhibit the proliferation of MCF7 cancer cell line on dose dependent manner. From these findings, the research was proved the classical use of Broccoli for the management of chronic illness as a modern nutraceutical.

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