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Effect of Gamma Irradiation on Antioxidant Activity of Phytochemicals in Selected Medicinal Plants

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Abstract— Medicinal plants have great importance for human health. The value of medicinal plants lies in some chemical substances having physiological role in human body; the most important of these are alkaloids, polyphenols and flavonoids. In the current study, the distribution of these moieties in three medicinal plant extracts belonging to different families was assessed. The radical scavenging activities of the plant extracts against 2, 2 –Diphenyl-1-picrylhydrazyl radical (DPPH) were determined through spectrophotometry. The effect of gamma irradiation was also observed on flavonoids and phenolic compounds. Results showed that antioxidant properties were significantly increased after irradiation. The study revealed an efficient effect of varying levels of gamma radiations, based on the pharmaceutical demand to enhance the accumulation and distribution of bioactive compounds such as phenolic and flavonoid compounds, fatty acids, as well as their antioxidant activities in the leaves of Viola odorata, roots of Nelumbonucifera (Gaertn) and Fruit of Carallumatuberculata.

Index Terms— 2, 2 –Diphenyl-1-picrylhydrazyl radical, gamma irradiation, antioxidant activity, alkaloids flavonoids, phenol.

1 INTRODUCTION

PLANTS have the ability to synthesize a wide variety of chemical compounds used to perform important biological functions and to defend against pathogens such as insects, fungi and herbivorous animals [1]. The use of herbs to treat disease is almost universal among non-industrialized societies, and is often more affordable than purchasing expensive modern pharmaceuticals. Chemical compounds in plants mediate their effect on the human body through processes identical to those already well understood for the compounds in conventional drugs. But on the other hand this enables herbal medicines to have harmful side effects [2]. The World Health Organization (WHO) estimates that 80 percent of the population of some Asian and African countries presently uses herbal medicine for primary health care. Studies in the United States and Europe have shown that their use is less common in clinical settings, but has become increasingly more common in recent years as scientific evidence about the effectiveness of herbal medicine has become more widely available [3].

All plants produce chemical compounds as part of their normal metabolic activities. These phytochemicals are divided into (1) primary metabolites such as sugars and fats, which are found in all plants; and (2) secondary metabolites found in a smaller range of plants, serving a more specific function [4].

For example, some secondary metabolites are toxins used to deter predation and others are pheromones used to attract insects for pollination. These secondary metabolites and pigments can have therapeutic actions in humans and can be refined to produce drugs, examples are inulin from the roots of dahlias, quinine from the cinchona, morphine, codeine from the poppy and digoxin from the foxglove [5].

Phytochemicals are chemical compounds that occur naturally in fruits, vegetables, beans, grains, and other plants. Each phytochemical has a variety of different plant sources with different proposed effects on the body. Some are responsible for color and other organoleptic properties, such as the deep purple of blueberries and the smell of garlic. Phytochemicals may have biological significance, for example carotenoids or flavonoids, but are not established as essential nutrients. There may be as many as 4,000 different phytochemicals [6]. Phytochemicals are commonly known as antioxidants, flavonoids, phytonutrients, flavones, isoflavones, catechins, anthocyanidins, isothiocyanates, carotenoids, allyl sulfides and polyphenols [7].

Gamma irradiation is a method of sterilization where products are exposed to gamma rays. The gamma rays, which are a form of electromagnetic radiation of very short-wave lengths, act as a source of ionizing energy that destroys bacteria and pests. The isotope Cobalt-60 is the most common source of gamma rays for irradiation processing. The Cobalt-60 is manufactured specifically for the irradiation process and is stored in specially designed chambers operating to strict standards and regulations [8]. Steritech's gamma irradiation process does not involve sufficient energy to make treated products radioactive. The product being treated does not come into contact with the Cobalt-60 and when the process is complete the energy does not remain in the product. Gamma irradiation is known as a 'Cold Process' as the temperature of the processed product does not increase significantly. It is also a chemical free process that is not reliant on humidity, temperature or pressure and can be applied to packaged goods [9].

Currently the effect of various doses of gamma radiation on different parameters of selected herbal ingredients. The post irradiation effects were analyzed on the proximate nutrients composition, phytochemical constituents such as total phenol and flavonoids in chosen herbal material. Antioxidant activity of herbal ingredient with DPPH and correlation of irradiation was estimated to determine the effect of radiation on solvent extractive values and to develop the safe range of gamma irradiation for sanitary mad Phytosanitary treatment.

2 Materials and Methods

Plant samples were collected from vicinity of National Institute of Food and Agriculture, Peshawar, Pakistan (NIFA). Only the specific parts of plants were sampled, like roots of Nelumbo, fruit of Carruluma and leaves of Viola. Fresh roots, stem bark, leaves and fruits of selected plants were collected. The selected parts of the plants were dried at room temperature. The dried samples were ground in free air circulation because in normal grinding the heat is produced which results in the component to dry. Thus, this study used the process of free air circulation. The plant material was air-dried at room temperature 25°C for two weeks, and the grinded to a uniform powder. The methanol extracts were obtained by macerating the powdered samples (5 g/100ml of methanol). The extracts were concentrated using a rotary evaporator under vacuum with the water bath set at 45 °C. Irradiation treatment was carried out for the samples at different doses 3, 6, 9 KGy using an experimental cobalt-60 Gamma source at the NIFA, Peshawar. The activity of the source was 8.5 KGy/hr and the energy 1.33 MeV. The irradiation time varied from 1 to 3 hours depending on the dose applied.

A 5 g of each plant sample was macerated using 100 ml of ethanol and was allowed to shake for 4 hours, then left for 72 hours. The extracts were filtered using Whatman no. 1 filter papers. The solvent was evaporated under reduced pressure using a rotary evaporator. The extracts were placed in Petri dishes and then air-dried until the solvent was completely evaporated. The different dried extracts were stored at 4°C. Then phenolic content, flavanoids and DPPH radical scavenging activity was determined. For filtration of both water and ethanol, the Whatman 42 filter paper was used. After filtration, two buffers one for pH '7' and other was for '4' pH. KCl were used. The pH was adjusted to pH '7' at temperature 23.5 and stop button was pushed to adjust pH to '4' at temperature 23.6 °C. Three observations were collected. The ethanolic sample was poured in petri dish and kept for incubation at 40 °C. Water was evaporated and the sample was extracted. For the extraction, the rotary vacuum evaporator machine was used. The extracted sample was weighted. For preparation of DPPH solution (0.1mM), 5 mg of extract was dissolved in 20 ml of methanol. The solution was kept in darkness to complete the reaction.

3 RESULTS

Accumulation of total phenolic content in the plant leaves of Viola. O was considerably affected by different levels of acute gamma irradiation ($p < 0.05$). The overall results demonstrated that leaves under 3 kGy and 6 kGy and 9 kGY of acute gamma irradiation exhibited higher content of total phenolic content as compared to Control. In case of

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Caralluma Tuberculata was, the overall results demonstrated that fruit under 3 kGy and 6 kGy and 9kGY of acute gamma irradiation exhibited lower content of total phenolic content as compared to Control. In Nelumbo Nucifera, results demonstrated that leaves under 3 kGy, 6 kGy and 9kGY of acute gamma irradiation exhibited higher content of total phenolic content compared to Control (Table 1).

In case of ethanolic extracts phenolic content was highest in VIOLA ODORATA at the dose of 9KGY compared to other doses and plant extracts. VIOLA ODORATA (Table 2). The flavonoid content in the leaves of VIOLA ODORATA was significantly high at the dose of 6KGY other extracts and radiation doses (Table 3). For ethanolic extracts the flavonoids content was high in control group of roots of Nelumbonucifera (Table 4). The moisture and ash percentage was maximum in the fruit extract of Caralluma T. Fat, protein and fiber percentage was maximum in irradiated Viola O leaf extract. Irradiated Nelumbu. N root extract showed maximum CH_6 percentage (Table 5).

TABLE 1

TOTAL PHENOLIC CONTENT IN THE LEAVES OF VIOLA ODORATA, ROOTS OF NELUMBONUCIFERA (GAERTN) AND FRUIT OF CARALLUMATUBERCULATA UNDER DIFFERENT DOSE LEVELS OF ACUTE GAMMA IRRADIATION

Replications	R1	R2	R3
V.O Cont H_2O	.269	.269	.269
V.O 3KGY H_2O	.304	.304	.304
V.O 6KGY H_2O	.354	.354	.354
V.O 9 KGY H_2O	.434	.434	.434
CT Cont H_2O	.297	.297	.297
C .T 3KGY H_2O	.251	.251	.251
CT 6KGY H_2O	.287	.286	.286
C.T 9 KGY H_2O	.273	.273	.273
N.N Cont H_2O	.236	.237	.237
N.N 3KGY H_2O	.237	.237	.238
N.N 6KGY H_2O	.242	.242	.243
N.N 9 KGY H_2O	.245	.245	.245

TABLE 3

TOTAL FLAVONOID CONTENT IN THE LEAVES OF VIOLA ODORATA, FRUIT OF CARALLUMATUBERCULATA AND ROOTS OF NELUMBONUCIFERA (GAERTN) UNDER DIFFERENT DOSE LEVELS OF ACUTE GAMMA IRRADIATION

Replications	R1	R2	R3
V.O Cont	.015	.015	.015
V.O 3KGY	.007	.007	.007
V.O 6KGY	.037	.037	.037
V.O 9 KGY	.026	.027	.027
C.T Cont	.028	.028	.028
C.T 3KGY	.020	.020	.020
C.T 6KGY	.008	.008	.008
C.T 9 KGY	.017	.017	.017
N.N Cont	.011	.011	.011
N.N 3KGY	.007	.007	.007
N.N 6KGY	.013	.013	.013
N.N 9 KGY	.009	.009	.009

TABLE 2

TOTAL PHENOLIC CONTENT IN THE LEAVES OF VIOLA ODORATA, ROOTS OF NELUMBONUCIFERA (GAERTN) AND FRUIT OF CARALLUMATUBERCULATA UNDER DIFFERENT DOSE LEVELS OF ACUTE GAMMA IRRADIATION OF ETHANOLIC SAMPLE

Replications	R1	R2	R3
V.O Cont H_2O	.334	.334	.334
V.O 3KGY H_2O	.381	.378	.377
V.O 6KGY H_2O	.356	.356	.356
V.O 9 KGY H_2O	.513	.512	.511
N.N Cont H_2O	.262	.270	.271
N.N 3KGY H_2O	.242	.242	.242
N.N 6KGY H_2O	.269	.269	.269
N.N 9 KGY H_2O	.313	.313	.313
CT Cont H_2O	.276	.276	.275
C .T 3KGY H_2O	.270	.270	.270
CT 6KGY H_2O	.260	.260	.260
C.T 9 KGY H_2O	.275	.275	.275

TABLE 4

TOTAL FLAVONOID CONTENT IN THE LEAVES OF VIOLA ODORATA, ROOTS OF NELUMBONUCIFERA (GAERTN) AND FRUIT OF CARALLUMATUBERCULATA UNDER DIFFERENT DOSE LEVELS OF ACUTE GAMMA IRRADIATION OF ETHANOLIC SAMPLE

Replications	R1	R2	R3
V.O Cont	.040	.040	.040
V.O 3KGY	.116	.115	.115

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V.O 6KGY	.120	.120	.120
V.O 9 KGY	.120	.121	.121
N.N Cont	.377	.377	.378
N.N 3KGY	.204	.204	.204
N.N 6KGY	.329	.330	.330
N.N 9 KGY	.324	.324	.324
C.T Cont	.070	.070	.070
C.T 3KGY	.027	.027	.027
C.T 6KGY	.048	.048	.048
C.T 9 KGY	.029	.029	.029

TABLE 5

CONCENTRATION OF DIFFERENT PHENOLIC COMPOUNDS IN THE LEAVES OF VIOLA ODORATA, ROOTS OF NELUMBONUCIFERA (GAERTN) AND FRUIT OF CARALLUMATUBERCULATA UNDER DIFFERENT DOSE LEVELS OF ACUTE GAMMA IRRADIATION

Plants	Radiation doses	Moisture content %	Ash %	Fat %	Protein %	Fiber %	CH_6 %
Viola Odorata	3	5.6	12.0	10.1	20.83	23.2	28.3
	6	5.4	12.2	10.4	22.92	23.7	25.4
	9	5.4	12.2	10.5	22.93	23.5	25.4
Nelumbu. N	3	5.2	5.5	7.9	7.2	11.7	62.5
	6	5.0	5.6	8.3	7.5	10.9	62.7
	9	5.0	5.6	8.1	7.4	11.8	62.1
Caralluma T	3	6.7	17.5	7.3	6.7	18.3	43.5
	6	6.7	17.6	7.9	7.5	17.9	42.9
	9	5.9	17.7	7.7	7.7	18.5	42.5

4 Discussion

The results indicated that the accumulation of total phenolic content in the plant leaves of Viola. O was considerably affected by different levels of acute gamma irradiation and it had a significant ($p < 0.05$) effect on total phenolic content of irradiated groups. The overall results demonstrated that leaves under 3 kGy and 6 kGy and 9kGY of acute gamma irradiation exhibited higher content of total phenolic content with values, as compared to Control. Total phenolic component in roots of Nelumbo Nucifera was considerably affected by different levels of acute gamma irradiation and it had a significantly higher ($p < 0.05$) total phenolic content of irradiated groups. The overall results demonstrated that leaves under 3 kGy and 6 kGy and 9kGY of acute gamma irradiation exhibited higher content of total phenolic content as compared to Control with respective values of .236.

The accumulation of total phenolic content in the Fruit of Caralluma Tuberculata was considerably affected by different levels of acute gamma irradiation except 5kGY dose and it had a significant ($p < 0.05$) effect on total phenolic content of irradiated groups. The overall results demonstrated that fruit under 3 kGy and 6 kGy and 9kGY of acute gamma irradiation exhibited lower content of total phenolic content as compared to control.

Previous studies demonstrated that various forms of irradiation influenced the phenolic and flavonoids content. Gamma irradiation (10 KGy) increased phenolic acid content in cinnamon and clove while phenolic content in nutmeg did not change [10]. Variyar et al, indicated that the free phenolic (aglycone) content of the soybean samples treated with gamma irradiation at levels ranging from 0.5 to 5 kGy increased [11]. The increment of total phenolic content and flavonoid under different levels of gamma irradiation could be ascribed to the release of these compounds from glycosidic forms and the degradation of larger compounds into smaller ones by gamma irradiation [12].

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