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Phytochemical screening and antioxidant potential of methanolic extract of *Annona squamosa, Citrus media* and *Prunus persica*

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Abstract

The present study was carried out in In-vitro antioxidant effects of the Methanolic leaf extracts of Annona squamosa, Citrus media and Prunus persica against 2,2-diphenyl-1-picrylhydrazyl and Nitric oxide radical. In DPPH method the extract of A. squamosa, C. media and P. persica displayed antioxidant activities with the IC_{50} values of 177.53 ìg/ml, 190.43 ìg/ml and 112.11 ìg/ml, respectively, but less than ascorbic acid (IC_{50} 42.73 ìg/ml). FIC_{Index} of combinations showed no synergistic effect of extracts. The extracts showed weak nitric oxide-scavenging activity in comparision to standard. Phytochemical screening of the plants showed the presence of flavonoids, alkaloids, carbohydrates, proteins, tannins and. Phenols. All plants did not contain protein while Annona squamosa and Prunus persica also showed the absence of alkaloids. Carbohydrates, tannins and phenols were similarly absent in Prunus persica. The data obtained in the in vitro models clearly establish the antioxidant potency of all extracts.

Introduction

There has an increasing interest in study of medicinal plants such as natural products in different part of world. Medicinal plants containing active chemical constituents with high antioxidant property play an important role in the prevention of various degenerative diseases. Antioxidants are one such substance which has capability to neutralized free radicals and their action. Antioxidants can interfere with the oxidation process by reacting with free radicals, chelating catalytic metals, acting as oxygen scavengers [1] prevent lipid autoxidation. Natural antioxidants like Vit C ,and Vit E, caratenoids and polyphenol like flavonoids are considered to be beneficial component from fruit and vegetables [2.3]. They are responsible for protective effects against different diseases e.g. Cerebrovascular Disease, Cancer, Arteriosclerosis the, Atherosclerosis, Heart Disease, Senility, Aging, Behcet's Disease, Crohn's Disease, Cataracts, Sunburn, Ulcers, Osteoporosis, Rheumatoid Arthritis, Diabetes Mellitus, Emphysema, Stroke [4]. Oxidation is essential to many living organisms for the production of energy to fuel biological process .Exogenous and endogenous metabolic process in animal body or in the food system might produced highly reactive free radicals, which are capable of oxidizing biomolecules, resulting in cell death and tissue damage. Free radical are produced in normal and or/ pathological cell metabolism. The free radicals (Super oxide, Hydroxyl radical and Nitric oxide) and their reactive species (Hydrogen peroxide, Hydrochloric acid) are generated in specific organelles of cell (Mitochondria and Microsome) under the normal physiological condition. It is important to study the antioxidant activity of locally available herbs using their active principles for the overall improvement of health. We further analyzed the free radical scavenging kinetic behavior of methanolic extracts of *Annona squamosa, Citrus media, Prunus persica*, and investigate their antioxidant performance of binary mixtures of to determine the type of interaction between them.

Materials and Methods

Materials

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Preparation of plant extracts

The medicinal plants used in this study, were collected from local region of Agra and Mathura district of country. The collected plant parts (*Annona squamosa, Citrus media, Prunus persica* leaves) were washed with clean water and allowed to shade dried for 2-3 weeks. The dried materials were crushed in an electric grinder to coarse powder. Crude plant extract was prepared by soxhlet extraction method as per Okeke *et al* [5]. About 100 gm of powder material was uniformly packed into a thimble and run in soxhlet extractor. It was exhaustible extracted with methanol for a period of about 48 hours or 22 cycles or till the solvent in the siphon tube of an extractor become color less. After that extracts were filtered with the help of filter paper and concentration of extract was done by vacuum rotatory evaporator (Heidolph, Germany) at set bath and cooling temperature of 35°C along with 147 bar vacuum pressure. Then all extracts were stored at 4°C for further detection of antioxidant activity and phytochemical analysis.

DPPH scavenging Test

Quntitative measurement of radical scavenging properties was carried out in a universal bottle by using some modified method [6]. The reaction mixture contained 1ml of test sample (MeOH as a blank) ans 1ml of .1mM DPPH in methanol. Standard antioxidant ascorbic acid was used for comparision or as a positive positive control. Discoloration was measured 517 nm after incubation for 30 min. Mesurement were taken at least in triplicate. DPPH radical concentration was calculated using the following equation.

$$\text{DPPH scavenging effect} = \frac{A_0 - A_1}{A_0} \times 100$$

Where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of sample. The actual decrease in the absorption in induced by test compound was compared with the positive control.

Nitric oxide scavenging activity

Nitric oxide scavenging activity was measured by the spectrophotometry method [7]. Sodium nitroprusside (5mmol) in phosphate buffered saline was mixed with a control without the test compound, but with an equivalent amount of methanol. Test solutions at different concentrations (7.8-1000 µg/ml) were dissolved in methanol and incubated at 25º C for 30 min. After 30 min, 1.5 ml of the incubated solution was removed and diluted with 1.5 ml of griess reagent (1% sulphanilamide, 2% phosphoric acid, and 0.1% naphthyl ethylenediamine dihydrochloriode). The absorbance of the chromophore formed during the subsequent coupling with naphthyl ethylenediamine dihydrochloriode was measured at 546 nm. IC_{50} values were calculated at different intervals for test samples and standard using Finney, 1962

Combination System

Synergistic and antagonist effect of plant extract was evaluated by the using the extract alone and different dose combinations to find out activity enhancement i.e. increase/decrease. For the binary mixtures (A + B) experimental data were transformed to fractional inhibitory concentration (FIC) as:

$$FIC_A = \frac{\text{Activity of compound A in the presence of B}}{\text{Activity of compound A individually}}$$

$$FIC_B = \frac{\text{Activity of compound B in the presence of A}}{\text{Activity of compound B individually}}$$

Subsequently, to establish if the binary mixtures tested are synergistic, antagonistic or additive, the fractional inhibitory concentration index (FIC_{index}) was calculated as: $FIC_{index} = FIC_A + FIC_B$ Data for doses points appearing below the additivity line are considered as synergic effects in a range of $FIC_{index} < 0.9$, additive effects in a range $0.9 < FIC_{index} < 1.1$ and antagonic effects for $FIC_{index} > 1.1$, according to [8].

Phytochemical screening of extracts

Extracts were tested for the presence of active principle such as tannins, flavonoides, alkaloides, glycosides, and proteins following standard procedure described by Debela [9] were used.

Results and Discussion

The DPPH antioxidant assay is based on the ability of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to be decolorized in the presence of antioxidants [10]. The DPPH radical contains an odd electron, which is responsible for the absorbance at 515-517 nm and also for visible deep purple color.

Table 1 shows the class of DPPH antioxidant capacity of the methanolic extracts of *Annona squamosa, Citrus media* and *Prunus persica*. The order of antioxidant capacity is as follows: *Prunus persica* (IC_{50} 112.11 ìg/

ml) > *Annona squamosa* (IC_{50} 177.53 ìg/ml) > *Citrus medica* (IC_{50} 190.43ìg/ml) (Fig-1). These Extracts showed no significant antioxidant capacity in comparision standard ascorbic acid (IC_{50} 42.73 ìg/ml), when these extracts used in combination FIC_{Index} represents (Table-2) no synergistic combination. With FIC_{Index} of 6.68, 2.09 and 1.27 A.squamosa + *C. media, C. media + P. persica* and *A.squamosa* + *P. persica* showed antagonistic effect of combination system.

Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reduction. Scavengers of nitric oxide complete with the oxygen, leading to reduced production of nitric oxide [11]. The griess assay used to determine the nitrite concentration is a suitable method for bio-guided fractionation of potential anti-inflammatory plant extracts [12]. The extracts of *Annona squamosa* and *Prunus persica* showed 50.2 and 43.6 % inhibition at the concentration of 1000 ìg/ml in nitric oxide-scavenging whereas, ascorbic acid showed 75.2 % inhibition on same concentration (Table-3). The IC_{50} value of Citrus *medica* was unable to determine.

The phytochemical screening of the plants studied showed the presence of flavonoids, alkaloids, glycosides, phenols, saponins and tannins (Table 4), *A. squamosa and P. Persia* showed the absence of alkaloids. *P. Persia* tested negative for the presence of carbohydrates and tannins and only *A. squamosa* tested negative for the presence of glycosides.

Conclusion

In conclusion, all plant extracts showed lowest antioxidant activity than standard ascorbic acid. When these extracts were used in combination no any synergistic combination was found.

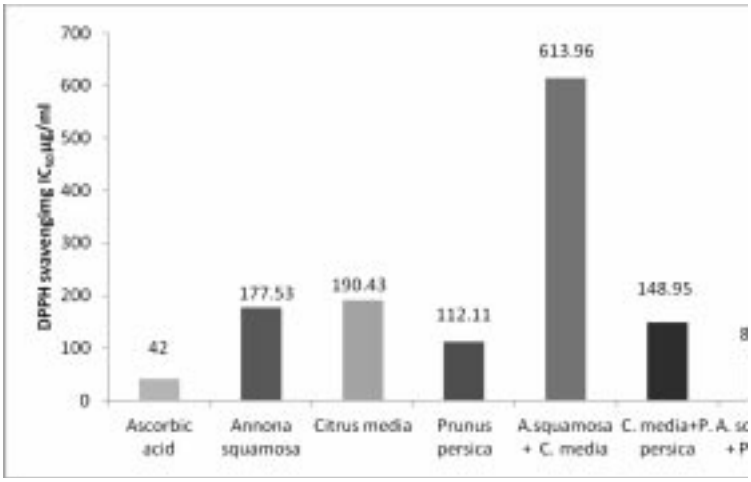

Fig-1 IC_{50} Values of DPPH scavenging activity of Plant extracts and Ascorbic acid.

Fig-1 IC_{50} Values of Nitric Oxide scavenging activity of Plant extracts and Ascorbic acid.

Table 1 : Percentages of DPPH scavenging of Plant extracts used alone and in combination.

Conc. (?g/ml)	Ascorbic Acid	*Annona squamosa*	*Citrus media*	*Prunus persica*	*A.* +
7.8	21.7±.036	19.5±.243	5.0±.096	15.7±.073	1
15.6	38.4±.036	25.8±.183	11.5±.055	17.9±.205	1
31.2	45.4±.073	28.3 ±.136	31.4±.073	26.5 ±.092	1
62.5	54.3±.036	32.0±.228	44.2±.138	31.2±.055	1
125	64.0±.055	45.3±.220	44.2±.218	47.2±.073	2
250	75.4±.055	61.4±.132	50.9±.353	68.2±.055	4
500	86.2±.092	66.0±.340	73.3±.111	80.5±.076	5
1000	93.4±.055	69.7±.235	76.2±.073	84.0±.073	5
IC_{50} (?g/ml)	42.73	177.53	190.43	112.11	

Table 2 : FIC_{Index} of combination of plant extracts.

Table 3 : Percentages of Inhibition in nitric oxide assay of plants extracts with standard.

Conc. (?g/ml)	Ascorbic Acid	*Annona squamosa*	*Citrus media*	*P p*
7.8	21.6±.055	11.5±.055	3.8±.145	9.
15.6	34.4±.055	16.9±.055	6.2±.088	14
31.2	41.5±.063	23.3 ±.055	7.1 ±.273	23
62.5	56.0±.092	26.8±.055	8.5±.088	25.
125	62.5±.128	35.5±.073	9.3±..020	34
250	66.4±.055	39.5±.055	10.7±.202	38
500	70.1±.055	47.8±.055	14.6±.120	40
1000	75.2±.073	50.2±.055	15.4±.025	43
IC_{50} (?g/ml)	63.94	729.54	ND	12

Table 4 : Phytochemical analysis of Plant extracts

References

1. **Shahidi F., Janitha P.K. and Wanasundara P.D.** 1992. Phenolic antioxidants. Crit. Rev. Food Sci. Nutr., 32: 67-103
2. **Gupta S. and Prakash** 2009 studies Indian on Indian green leafy vegetables for their antioxidant activity, Plant food Hum Nutr,64: 39-45.
3. **Serteser A., Kargýoglu M. , Gok V. , Bagci Y. , Ozcan M. and Arslan D. ,** 2009. Antioxidant properties of some plants growing wild in Turkey. Grasasy Aceites, 60: 147-154.
4. **Sharma H. and Clark C.**, 1998. An excerpt from the medical textbook Contemporary Ayurved., Edinburgh: Churchill Livingstone, ISBN: 0 443 05594 7.
5. **Okeke M.I., Iroegbu C.U., Eze E.N., Okoli A.S. and Esimone C.O.** 2001.Evaluation of extracts of the roct of *Landolphia owerrience* for antibacterial activity. J.Ethnopharmacol. 78:119-127.
6. **Oktay M., Gulcin I. and Kufrevioglu O.I.** 2003. Determination of in vitro antioxidant activity of fennel (Foeniculum vulgare) seed extracts. Lebensmittel-Wissenschaft und-Technologie, 36: 263-271.
7. **Madan N.P., Raghavan G., Singh A.K., Palpu R. P.** 2005. Free radical scavenging potential of *Saussarea costus*, *Acta Pharm*, 55: 297-304.
8. **Santiesteban-Lopez A., Palou E. and Lopez-Malo A.** 2007. Susceptibility of foodborne bacteria to binary combinations of antimicrobials at selected aw and pH. *Journal of Applied* Microbiology, 102, 486–497.
9. **Debela A.** 2002 Manual for phytochemical screening of medicinal plants Ethaiopian Health and Nutrition Research Institute, *Ethiopia,* 35-47.
10. **Kumarasamy Y., Byres M., Cox P. J., Jaspars M., Nahar L. and Sarker S. D.** Screening seeds of some Scottish plants for free-radical scavenging activity, Phytotherapy Research, 2007; 21: 615-621.
11. **Govindarajan R., Rastogi S., Vijaykumar M., Shirwaikar A., Rawat A.K. and Mehrotra S.,** 2003. Studies on antioxidant activities of Desmodium gangeticum. Bio. Pharm Bull, 26: 1424-1427.
12. **Dirsch V.M., Stuppner H. and Vollmer A.M.** 1998. The griess assay: Suitable for a bio-guided fractionation of anti-inflammatory plant extracts? Planta Medica, 64: 423-426.

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