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ANTIOXIDANTS AND ANTIMICROBIAL ACTIVITY OF SOME MEDICINALLY IMPORTANT PLANTS

Sumit Sheoran

PDM university Bahadurgarh, Haryana, Hisar 125001, India

Abstract

In the human body, there are highly reactive species or uncharged molecules having unpaired valance electron formed by exothermic and endothermic chemical metabolic reactions that are having the capability to oxidize organic molecules. All organisms have inbuild systems consist of organic catalysts such as catalases, glutathione reductase, and glutathione peroxidase, etc which can compensate for the effects of free radicals and other oxygen reactive species. To Check the antioxidant activity I perform TPC, TFC, DPPH, CAT, GSH, GST. I take fruits of medicinal important plants i.e. Malus domestica (Apples), Phyllanthus emblica (Amlas), Vitis vinifera (graphs), Allium sativum (Garlic), Syzygium aromaticum (Cloves) and Solanum lycopersicum (Tomatoes).

Keyword: Antioxidants, Antimicrobial, Medicinal plants, Biotechnology, Biochemistry,Life sciences

1.INTRODUCTION

In the human body, there are highly reactive species or uncharged molecules having unpaired valance electron formed by exothermic and endothermic chemical metabolic reactions that are having the capability to oxidize organic molecules i.e. DNA, Nucleic acid, Protein etc and can generate different diseases like cancer, cirrhosis, etc.(1,2)

All organisms have inbuild systems consist of organic catalysts such as catalases, glutathione reductase, and glutathione peroxidase, etc which can compensate for the effects of free radicals and other oxygen reactive species. The human body remains in a neutral state until the oxygen reactive species are controlled by the inbuilt system(immune system). An increase in oxygen reactive species due to loss or decrease in the antioxidants present in the body produce oxidative stress and can be the reason for DNA damages, Mutation, Cancer in the body(3) Antioxidants neutralize the effects of free radicals. (4)

In past decades, several biologist or biochemist worked and found some medicinal plants which are helpful to increase the antioxidants contained in the human body and to reduce the effects of oxygen reactive species. Unlike the 20th century, many modern biochemists show their consent towards natural products or medicinal plants in the past few years.(5,6)

The objective of my works to evaluate the comparitive antioxidant activities of Apple fruit(Malus domestica), Tomato fruit(Phyllanthus emblica), garlic(Allium sativum),Grapes(Phyllanthus emblica), and clove(Syzygium aromaticum), Datura(Datura stramonium), bargad bark(Ficus benghalensis), Pipli(Exbucklandia populnea), Castor(Ricinus communis), Crown Flower(Calotropis gigantea), Crown fruit(Calotropis gigantea), and Monkey fruit(Limonia acidissima).

2.MATERIAL & METHOD

2.1. SAMPLE COLLECTION:

Malus domestica (Apples),
Phyllanthus emblica (Amlas),
Vitis vinifera (graphs),
Allium sativum (Garlic),
Syzygium aromaticum (Cloves) and
Solanum lycopersicum (Tomatoes)

Fruit of all these plants were collected in the month of Dec. and January at morning and evening. I have been collected these plants from various botanical gardens and verified by Dr. Ravi prof. Hindu college.

2.2. Plant drying:

All the fresh fruits of the plants are firstly separated then washed with distilled water and then cut in pieces and put in hot air oven for one weak for drying.

2.3. Preparation of extract:

After drying they are collected and cut in very small pieces of apple and Grapes and then make powders of all fruits separately using grinder and put in air tight bottles for further uses.
After that weigh 10gmsof each fruit powder and make a packet separately.
After that we use soxhlet extraction unit for preparing extraction. We add 350ml of ethanol and put 10gms of powder and put in soxhlet extraction unit and starts the process. It takes 1-2 days for each plant. After that the liquid after this process filled in petri plates and those petri plates put in hot air oven for drying and then that drying extraxt is put in ependoffs for further uses. This process is same for all plants.

3.DPPH

2,2-Diphenyl-1-picrylhydrazyl (DPPH) is a stable free radical. DPPH free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in ethanol. DPPH assay measures compounds that are radical scavengers.

- Ethanol and Distilled water served as blank
- DPPH and Distilled water served as Control
- 1ml DPPH solution added to the pure green tea and mix green tea samples
- Observed absorbance at 517 nm

$$\%\text{Inhibition} = \frac{\text{Abs(Control)-Abs(Sample)}}{\text{Abs(Control)}}$$

Table

No	Sample	DW	Ethanol	DPPH	OD
1	-	1ml	1ml	-	-
2	-	1ml	-	1ml	0.142
3	Apple	-	-	1ml	0.139
4	Amla	-	-	1ml	0.060
5	Grape	-	-	1ml	0.127
6	Clove	-	-	1ml	0.071
7	Garlic	-	-	1ml	0.120
8	Tomato	-	-	1ml	0.126
9	Datura	-	-	1ml	0.105
10	Crown flower	-	-	1ml	0.069
11	Pipli	-	-	1ml	0.271
12	Castor	-	-	1ml	0.179
13	Bargad bark	-	-	1ml	0.218
14	Monkey fruit	-	-	1ml	0.031
15	Crown leaf	-	-	1ml	0.304

3.1. Result-

%Inhibtion of sample = Apple = 0.0211
Amla = 0.577
Grape = 0.105
Clove = 0.5
Garlic = 0.154
Tomato = 0.112
Datura = 0.66
Crown leaf = 0.016
Pipli = 0.122
Castor leaf = 0.421
Bargad bark = 0.294
Crown flower= 0.776
Monkey fruit = 0.899

4.TOTAL FLAVONOID CONTENT

4.1. Principle-

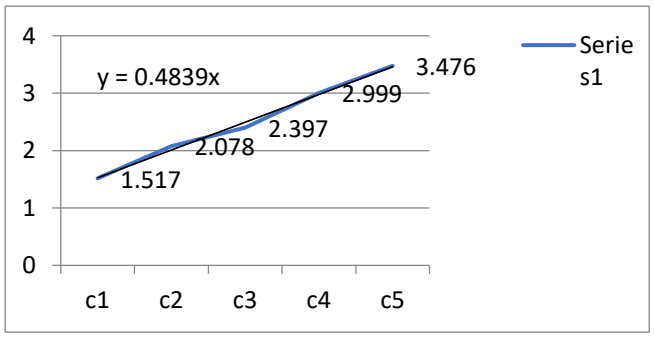
Total flavonoid content of samples were determined by aluminium chloride colorimetric method. In this we use 10% AlCl3, Methanol, Quercetin,1M Potassium acetate , DW
Prepared 1mg/ml quercetin stock solution and prepared different concentrations from 10 µg/ml to 200µg/ml.Blank consist of all the reagents except quercetin 1 ml each pure and mix green tea were taken as samples. 3 ml methanol was added to the tube. 200 µl AlCl3 was added.200 µl Potassium acetate was added.3 ml DW was added. Incubated at room

temperature for 30 min. and at last measure the Absorbance at 420 nm

Table-

No	Quercetin	DW	Methanol	$AlCl_3$	Potassium acetate	DW	Incubation	OD
1	-	1000 µl	3ml	200 µl	200 µl	3ml		-
2	10 µl	990 µl	3ml	200 µl	200 µl	3ml		1.517
3	50 µl	950 µl	3ml	200 µl	200 µl	3ml		2.078
4	100 µl	900 µl	3ml	200 µl	200 µl	3ml	30 mins	2.397
5	150 µl	850 µl	3ml	200 µl	200 µl	3ml	at room	2.999
6	200 µl	800 µl	3ml	200 µl	200 µl	3ml	temperature	3.476
7	1000µl (Pure)	-	3ml	200 µl	200 µl	3ml		
8	Tomato	-	3ml	200 µl	200 µl	3ml		0.848
9	Amla	-	3ml	200 µl	200 µl	3ml		2.777
10	Grape	-	3ml	200 µl	200 µl	3ml		2.221
11	Garlic	-	3ml	200 µl	200 µl	3ml		1.275
12	Apple	-	3ml	200 µl	200 µl	3ml		2.078
13	Clove	-	3ml	200 µl	200 µl	3ml		3.496
14	Datura	-	3ml	200 µl	200 µl	3ml		0.895
15	Crown leaf	-	3ml	200 µl	200 µl	3ml		2.026
16	Pipli	-	3ml	200 µl	200 µl	3ml		0.401
17	Castor	-	3ml	200 µl	200 µl	3ml		1.943
18	Bargad bark	-	3ml	200 µl	200 µl	3ml		1.252
19	Crown flower	-	3ml	200 µl	200 µl	3ml		0.542
20	Monkey fruit	-	3ml	200 µl	200 µl	3ml		0.663

4.2. Graph:



4.3. Result-

Total flavonoids content

1. Apple 4.294
2. Amla 5.738
3. Grape 4.589
4. Tomato 1.752
5. Lounge 7.22
6. Garlic 2.634
7. Datura 1.849
8. Pipli 0.828
9. Crown leaf 4.186
10. Castor 4.0152
11. Bargad bark 2.587
12. Crown flower 1.120
13. Monkey fruit 1.370

5.TOTAL PHENOL CONTENT

5.1. Principle-

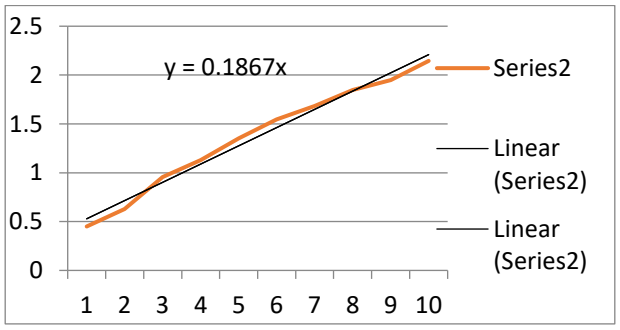
Phenolic Quantification Assay is based on Folin-Ciocalteu method. We requires Quercetin(1mg/ml), 20% NaNO3, 0.5 N FCR, DW.

After this we take 1 ml pure and mix green tea sample and added to the tube. Quercetin as standard from 100 µg/ml to 1 mg/ml. 100 µl FCR was added. Incubated for 10 min. at room temperature and add 2.5 ml NaNO3 and Incubated at room temperature for 30 min. and take Absorbance at 760 nm.

Table-

No	Quercetin	DW	FCR	Incubation	20% $NaNO_3$	Incubation	OD
1	-	1000 µl	100 µl		2.5ml		-
2	100 µl	900 µl	100 µl		2.5ml		0.330
3	200 µl	800 µl	100 µl		2.5ml		0.630
4	300 µl	700 µl	100 µl	10 mins	2.5ml	30 mins	0.955
5	400 µl	600 µl	100 µl	at room	2.5ml	at room	1.129
6	500 µl	500 µl	100 µl	Temperature	2.5ml	temperature	1.354
7	600 µl	400 µl	100 µl		2.5ml		1.545
8	700 µl	300 µl	100 µl		2.5ml		1.683
9	800 µl	200 µl	100 µl		2.5ml		1.848
10	900 µl	100 µl	100 µl		2.5ml		1.949
11	1000 µl	-	100 µl		2.5ml		2.145
12	Apple 1ml	-	100 µl		2.5 ml		0.184
13	Amla 1ml	-	100 µl		2.5ml		0.264
14	Grape 1ml	-	100 µl		2.5ml		0.139
15	Tomato 1ml	-	100 µl		2.5ml		0.063
16	Lounge 1ml	-	100 µl		2.5ml		0.353
17	Garlic 1ml	-	100 µl		2.5ml		0.021
18	Datura	-	100 µl		2.5ml		0.632
19	Crown leaf	-	100 µl		2.5ml		1.585
20	Pipli	-	100 µl		2.5ml		0.862
21	Bargad bark	-	100 µl		2.5ml		1.592
22	Crown flower	-	100 µl		2.5ml		1.019
23	Monkey fruit	-	100 µl		2.5ml		1.286
24	castor	-	100 µl		2.5ml		1.442

5.2. Graph



5.3. Result:

Y = MX+C

Apple	0.985
Amla	1.414
Grape	0.744
Lounge	1.890
Tomato	0.377
Garlic	0.112
Datura	3.385
Pipli	4.617
Castor	7.723
Crown flower	5.457
Crown leaf	8.489
Bargad bark	8.527
Monkey fruit	6.888

6.CATALASE ASSAY

6.1. Principle-

Catalase causes rapid decomposition of hydrogen peroxide to water.

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

We required

0.01M Phosphate buffer, pH 7.0 , 0.2 M Hydrogen peroxide Stock dichromate / acetic acid solution: Mixed 5 % potassium dichromate with glacial acetic acid (1:3 by volume). Working dichromate/acetic acid solution: The stock was diluted to 1:5 with water to make the working dichromate / acetic acid solution, DW.

After this we make assay mixture which contained 0.5 ml of H_2O_2, 1 ml of buffer and 400 µl of water. Add 200 µl of the enzyme to initiate the reaction. After 60 seconds add2 ml of the dichromate / acetic acid reagent. In control tube the enzyme was added after the addition of the acid reagent after that tubes were then heated for 10 min and then color developed was read at 610 nm. The activity of catalase was expressed as µmoles of H_2O_2 decomposed / min / mg protein.

Table

CONTROLS	OD	SAMPLES	OD
Apple	0.242	Apple	0.445
Amla	0.283	Amla	0.384
Grape	0.248	Grape	0.328
Garlic	0.168	Garlic	0.242
Tomato	0.176	Tomato	0.207
Clove	0.117	Clove	0.426
Datura	0.164	Datura	0.188
Crown leaf	0.382	Crown leaf	0.539
Pipli	0.125	Pipli	0.196
Castor	0.269	Castor	0.408

Bargad bark	0.384	Bargad bark	0.457
Crown flower	0.103	Crown flower	0.179
Monkey fruit	0.192	Monkey fruit	0.209

6.2. Result:

Apple = 1.15mg/dl
Amla = 2.16mg/dl
Grapes = 1.63mg/dl
Clove = 2.50mg/dl
Garlic = 0.61mg/dl
Tomato = 0.35mg/dl

7.GIUTATHIONE S-TRANSFERASE ASSAY(GST)

7.1. Principle

Glutathione S Transferase (GST) is an enzyme involved in detoxification of a wide range of compounds and is involved in reducing free radical damage in red blood cells. Identification of GST is done by western blotting or more easily by enzymatic assay. Enzyme Reaction: Glutathione –SH + CDNB -> Glutathione –S-CDNB The reaction is measured by observing the conjugation of 1-chloro, 2,4-dinitrobenzene (CDNB) with reduced glutathione (GSH). This is done by watching an increase in absorbance at 340 nm. One unit of enzyme will conjugate 10 nmol of CDNB with reduced glutathione per minute at 25ºC.

GST activity= [(Adjusted abs340/min)/0.0096 µM-1/cm]x (1ml/0.1ml) x any sample dilution

We required 100 mM CDNB (in ethanol), 100 mM Reduced Glutatione(GSH) (in ethanol), PBS, pH 6.5.
After that we make Assay cocktail (2 ml) in this we add 1.96 ml PBS, 20 µl CDNB, 20 µl GSH. After that for each sample and a blank, placed 1.8 ml of enzyme cocktail into cuvettes and Incubated at 30ºC in spectrophotometer for 5 min. To the blank cuvet added 100 µl PBS and in control or sample we add 100 µl of sample into the cuvets and take their Absorbance at 340 nm.

7.2. Result-

- Pure sample = 1467.7 U/ml
- Mix sample = 1188.5 U/ml

8.REDUCED GLUTATHIONE ASSAY(GSH)

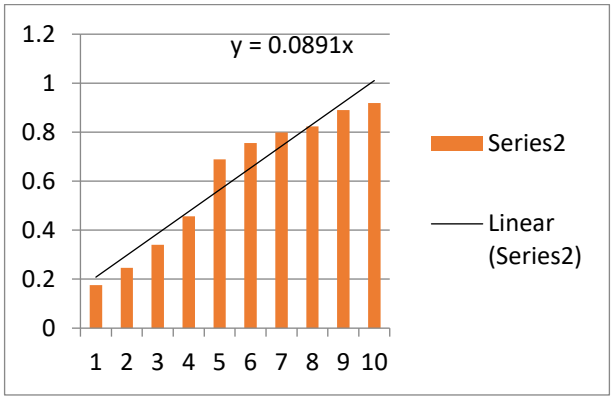
8.1. Principle-

Reduced glutathione (L-γ-glutamyl-L-cysteinylglycine), a tripeptide, serves as a key antioxidant in animal, plant, fungi and bacteria by providing free thiol. Glutathione exist in reduced (GSH) and oxidized (GSSG; gluthathione disulphide) forms in cells and tissues, Reduced glutathione on reaction with DTNB (5,5'-dithiobis nitro benzoic acid)produces a yellow coloured product that absorbs at 412nm. We required TCA (5%), Phosphate buffer (0.2M, pH 8.0), DTNB (0.6mM in 0.2M phosphate buffer), Standard GSH (10nmoles/ml of 5% TCA). Standard GSH is prepared 1µmole and GSH concentrations were made up to 1.0ml with 0.2M sodium phosphate buffer (pH 8.0) then add 200 µl of freshly prepared DTNB solution 1ml buffer was again added to the tubes and the yellow colour is developed and measure in a spectrophotometer at 412nm. The values are expressed as nmoles GSH/g sample.

Table-

No	GSH	Buffer	DTNB	Buffer	OD
0	-	1ml	200 µl	1ml	-
1	100 µl	900 µl	200 µl	1ml	0.176
2	200 µl	800 µl	200 µl	1ml	0.246
3	300 µl	700 µl	200 µl	1ml	0.340
4	400 µl	600 µl	200 µl	1ml	0.456
5	500 µl	500 µl	200 µl	1ml	0.689
6	600 µl	400 µl	200 µl	1ml	0.756
7	700 µl	300 µl	200 µl	1ml	0.799
8	800 µl	200 µl	200 µl	1ml	0.823
9	900 µl	100 µl	200 µl	1ml	0.890
10	1 ml	-	200 µl	1ml	0.919

8.2. Graph:



8.3. Result-

Pure sample =

1. Apple = 4.668 nmoles GSH/g sample
2. Amla = 6.734 nmoles GSH/g sample
3. Grape = 6.722 nmoles GSH/g sample
4. Tomato = 0.280 nmoles GSH/g sample
5. Clove = 1.885 nmoles GSH/g sample
6. Garlic = 0.381 nmoles GSH/g sample
7. Datura = 1.850 nmoles GSH/g sample
8. Crown leaf= 0.863 nmoles GSH/g sample
9. Pipli= 0.896 nmoles GSH/g sample
10. Castor = 2.151 nmoles GSH/g sample
11. Bargad bark = 2.251 nmoles GSH/g sample
12. Crown flower = 1.452 nmoles GSH/g sample
13. Monkey fruit = 1.542 nmoles GSH/g sample

9.RESULTS AND CONCLUSION:

In my work, I have collected several medicinal plants and evaluate for their potency to increase antioxidants in the human body by biochemical and chemical assays. Although the ethanolic contents showed a degree of antioxidant activity, there were considerable differences among different plants. There are many seasons and a large area that is used for growing plants so many species are found. So many biochemists used that plant extracts for their work and research (to known about their medicinal properties). (7) I collected some plants from nearby areas and tested for their phenolic and flavonoids content and their capacity to reduce or neutralize the effect of free radicals.

The presence of phenolic and flavonoids contents in the plants was confirmed by TPC and TFC method. The antioxidant capacity was measured by DPPH, GSH, GST, and Catalase.

The highest flavonoid content is found in CLOVEs, AMLA, AND GRAPES and others are less or very low content. While the highest phenolic content was observed in BARGAD BARK, MONKEY FRUIT and CROWN LEAF and others are less or very low.

From that result, I conclude that plants are very helpful to increase the number of antioxidants.(8) And they also have many other secondary metabolites, many other medicinal properties have been explained i.e. anticancer, antimicrobial, etc.(9.)

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