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RESEARCH ARTICLE

Anti-Oxidant Potential of some Herbal Drugs: A Bioactivity Guiding approach for Chronic Diseases

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ABSTRACT:

Herbal drugs are gaining momentum and worldwide acceptability for their importance in cure with safety, which has recently recognized and accepted globally during the COVID-19 pandemic. The therapeutic potential of plant-based traditional drugs in chronic diseases generally belongs to their combating effects on oxidative stress. Therefore, traditional herbal drugs can be screened out by assessing their anti-oxidant potential before performing their pre-clinical or clinical studies. The current study has demonstrated the *in-vitro* antioxidant potential of eight traditional medicinal plants by using five worldwide acceptable methods. The antioxidant potential was tested by evaluating total phenolic contents and reducing capacity along with the determination of DPPH, Nitric oxide, and Superoxide anionic free radical scavenging methods in eight traditional medicinal plants [viz. *Emblica officinalis* Linn., *Amomum subulatum* Roxb., *Coriandrum sativum* Linn., *Borago officinalis* Linn., *Cinnamomum cassia* Blume (leaves), *Nardostachys jatamansi* DC, *Crocus sativus* Linn. and *Santalum album]*. The correlation has been established among the results obtained, which can be considered as evidence of the method's reliability. The total phenolic contents have been estimated in all drug samples and amla was found to contain maximum, whereas sandal had minimum phenolic contents. The results of the reducing power method were compiled by taking three readings and presented with standard deviation. The results against DPPH, Nitric oxide, and superoxide free radicals were demonstrated by calculating % inhibition and subsequently by calculating IC_{50} values, respectively. The proposed methodology can be adopted to screen out the bioactivity-guided fractions as well as different plant samples before going to perform final in-vivo/preclinical or clinical studies for chronic diseases. The results of the study are encouraging and evidently complementary when correlations have been established among the results obtained by different methods.

KEYWORDS: *In-vitro* antioxidant activity, DPPH method, Free radicals, Scavenging, Total phenolic contents.

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INTRODUCTION:

Over the last ten years, and especially in light of the Covid-19 pandemic, the use of herbal remedies for therapeutic purposes has gained significant traction worldwide. The World Health Organization (WHO) noted that due to its superior cultural acceptability, more body compatibility, and less adverse effects, herbal medicine continues to be the primary source of primary healthcare for the majority of the world's population. The world's health has benefited greatly from medicinal plants. It is also noticed that about 25 – 30% of all

modern medicines are directly or indirectly derived from higher plants[1]. The "free radicals" or oxidative stress have been evidently discussed to damage all important biochemical components and micronutrients of the human body. Due to this it is the one of the most acceptable etiology of chronic illnesses in the human body. Human body contains some enzymes, antioxidants exists naturally or from the diet to combat the oxidative stress. Some natural free radical scavengers like vitamins A, C, and E, and carotenoids are also very much valuable to save our body from such oxidative stress and free radicals[2]. It has been observed by Professor L. Packer and his colleagues how carotenoids interact with vitamins C and E. Low-density lipoprotein was found to be shielded from oxidative damage by beta-carotene, even in the presence of low vitamin E levels. The antioxidants may act synergistically and offer a combined protection rather than an individual effect whereas herbal antioxidants such as phenols and bioflavonoids can potentiate antioxidant property of vitamins. When consumed in combination with vitamins C and E, the bioflavonoid rutin can enhance their ability to scavenge radicals more effectively[3]. The phytoconstituents and non-vitamin/mineral compounds that scavenge radicals constitute the foundation for the antioxidant factors present in plants. Certain plant species or combinations of herbs utilized in formulations have the ability to scavenge superoxide and function as antioxidants; these compound groups have the capacity to create cell-protective action through multiple biochemical mechanisms. The various classes of phyto-constituents, such as polyphenols, thiols, carotenoids, retinoids, carbohydrates, trace metals, terpenes, tocopherols, and the breakdown products of glucosinolates (isothiocyanates, indoles, and dithiothiols), can be categorized according to their chemical structure. The scavenging actions of polyphenols on active mutagens and carcinogens are even one of the modes of action of cancer-protective substances found in fruits and vegetables that prevent the onset of cancer[4]. Plant antioxidants are particularly important in the prevention and treatment of illness. Silymarin, a well-known hepatic antioxidant, is derived from the milk thistle plant, *Silybum marianum*, and it prevents liver damage by scavenging free radicals[5].

Some polyherbal Unani formulations give vitality and strength to the heart and it has been scientifically demonstrated that antioxidant intake and occurrence of various cardiovascular diseases have a strong relation. Several scientists have worked on these plants and have shown in their studies that they are rich source of substances that have several therapeutic properties including cardio-protection and have antioxidant effects as well[6-7]. In our present research work, we have to study *in-vivo* cardio-protective activity of medicinal plants by using doxorubicin induced cardio-toxicity in rodent model. Eight medicinal plants have been selected from the literature of the Unani Systems of medicine and assessed for their *in-vitro* anti-oxidant activity by using previously available and established methods. *Emblica officinalis* Linn., *Amomum subulatum* Roxb., *Coriandrum sativum* Linn., *Borago officinalis* Linn., *Cinnamomum cassia* Blume (leaves), *Nardostachys jatamansi* DC, *Crocus sativus* Linn. and *Santalum album* have been demonstrated for the estimation of total phenolic contents, evaluation of reducing power, and DPPH, Nitric oxide and Superoxide anionic free radical scavenging activity. The results obtained have been utilized to finalize the best plant for the further *in-vivo* study. A very first time correlation among the results obtained from different methods have also been established, which is supportive and encouraging.

METHODOLOGY:

Total phenolic contents:

Powdered samples that were precisely weighed were placed in a 50mL volumetric flask and allowed to reach a volume of 50mL using sonication with 40mL of double-distilled water for duration of 15 min.

Using catechin as a reference phenolic element, the Folin–Ciocalteau (F.C.) method was used to estimate the overall phenolic contents[8-9]. Distilled water was used to generate various dilutions ranging from 20 to 100µg mL^{-1} of standard catechin. A test tube was filled with one 0.5mL standard dilution per subject. The test tubes were filled with 4.0mL of 1M sodium carbonate (Na2CO3) solution and 5.0mL of F.C. reagent (10%). Additionally, 0.5mL of each sample extract was placed in a test tube, to which corresponding reagents were added, and it was left for 15min. The blue color's appearance was observed. At 765nm, the absorbance was measured in relation to a blank solution that contained 4.0mL of Na2CO3 solution, 5.0 mL of F.C. reagent, and 0.5mL of methanol. The linear regression equation was numerically determined by plotting the calibration curve following the measurement of the absorbance of standard dilutions. The standard calibration curve was utilized to calculate the phenolic content concentration in the samples.

Reducing power method:

All samples' reduction power was calculated using the previously defined techniques[10-11]. Ascorbic acid, a standard antioxidant, and various doses (5.0–25µg mL–1) of drug extracts were combined with 1.0 mL of distilled water, 2.5mL of 0.2M phosphate buffer (pH 6.6), and 2.5mL of 10% potassium ferricyanide [K3Fe (CN)6]. After 2.5mL of 10% trichloroacetic acid was added, the mixture was incubated at 50°C for 20 minutes. It was then centrifuged for 10 minutes at 3000 rpm. After the solution's upper layer (2.5mL) was

removed, it was combined with 0.5mL of 0.1% ferric chloride and 2.5mL of distilled water. At 700nm, the absorbance was measured. More reduction was indicated by the reaction mixture's increased absorbance. As a reference standard, ascorbic acid was utilized, and phosphate buffer (pH 6.6) served as the blank solution.

DPPH free radical scavenging method:

The stable radical DPPH method was used to assess the methanolic extract of all samples' ability to scavenge free radicals[12]. In methanol, DPPH solution (0.004%, w/v) was made. To create the stock solution (1.0mg mL-1), the methanolic extracts of each medication were combined with methanol. Test tubes containing freshly made DPPH solution and sample extracts were filled with 1.0mL of serial dilutions (5.0 - 25µg mL-1) in each test tube. As a reference standard, ascorbic acid was dissolved in methanol to create a stock solution with a concentration of 1.0mg mL-1. Serial dilutions of the stock solution (5.0 - 25µg mL-1) were then created in the same manner. After ten minutes, the absorbance was measured at 515nm. A control sample was made with the same volume but no extract and ascorbic acid as a reference. The blank was 95% methanol. The following formula was used to calculate the DPPH free radical's percent scavenging activity:

$$\% \text{ Inhibition} = [(A_0 - A_1) / A_0] \times 100$$

Where, A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance of the extract or standard.

Nitric oxide scavenging method:

All sample extracts' nitric oxide radical scavenging activity was assessed using the technique described in reference 13. The Griess-Illosvoy reaction can be used to measure the amount of nitrite ions that are produced when sodium nitroprusside is added to an aqueous solution at physiological pH. Nitric oxide is generated spontaneously by the reaction between nitric oxide and oxygen. Because nitric oxide scavengers compete with oxygen for their resources, nitric oxide synthesis is reduced. After mixing 0.5mL of each sample extract with ascorbic acid (a standard antioxidant) at different concentrations (5.0-25µg mL-1), 2.0mL of 10mM sodium nitroprusside in 0.5 mL phosphate buffer (pH 7.4) was added. The mixture was then incubated at 25ºC for 150minutes. After extracting 0.5mL from the mixture that had been incubated, it was combined with 1.0mL of sulfanilic acid reagent (33% in 20% glacial acetic acid, w/v) and allowed to sit at room temperature for five minutes. Lastly, 1.0mL of 0.1% w/v naphthylethylenediamine dihydrochloride was combined and allowed to sit at room temperature for 30 minutes. As a positive control, the absorbance of standard solutions of ascorbic acid treated in the same manner with Greiss reagent was used to measure the absorbance of the chromaphore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthylethylenediamine. The measurement was made at 540nm. All samples' nitric oxide radical scavenging activity was determined using a method comparable to the DPPH approach.

Super oxide anionic scavenging method:

Using the previously established methodology[14] the scavenging activity of each sample against superoxide anion radicals was determined. In a non-enzymatic phenazine methosulfate nicotinamide adenine dinucleotide (PMS-NADH) system, the reaction between PMS, NADH, and oxygen produced superoxide anions. Nitroblue tetrazolium (NBT) reduction was used as an analytical method.

3.0mL of phosphate buffer (100mM, pH 7.4), 0.75mL of NBT (300µM) solution, 0.75mL of NADH (936µM) solution, and 0.3mL of various concentrations (10-50µg mL-1) of all sample extracts were used for the spectroscopic examination. The mixture was mixed with 0.75mL of PMS (120µM) to start the reaction. A spectrophotometer was used to measure the absorbance at 560nm following 5.0 minutes of room temperature incubation. Super oxide anion scavenging activity was computed using a method akin to the DPPH approach.

Correlation between different *in-vitro* methods:

The correlation has been established among the results obtained from all methods which are used to assess the antioxidant efficacy of the drugs. The results of free radical scavenging efficacy were compared as nitric oxide scavenging efficacy compared with DPPH free radical and superoxide free radical scavenging efficacy. The radical scavenging efficacy was also compared with the total phenolic contents of respective drugs.

RESULTS:

Total Phenolic contents:

The primary evidence of the plant's antioxidant efficacy is the existence of phenolic components, which may directly contribute to their antioxidant qualities. Numerous research published recently indicate that phenolic compounds may have an inhibitory effect on human carcinogenesis and mutagenesis when consumed in conjunction with a diet heavy in fruits and vegetables[15]. The Folin-Ciocalteu method was used to determine the total phenolic contents of all samples, and the findings are shown as %w/w in Table 1 and Fig. 1A. The maximum contents were found in amla (20.82, %w/w) then in saffron (3.49, %w/w), jatamansi (4.12, %w/w) and amomum (2.79, %w/w). The minimum contents were observed in sandal (0.96, %w/w) and cassia leaves (1.04, %w/w).

Reducing power assay:

The presence of reductants, which have demonstrated antioxidative potential by disrupting the free radical chain and donating a hydrogen atom[16-17], is often required for a chemical to have the ability to reduce. The Fe^{3+}/ferricyanide complex is reduced to the ferrous form in the methanol extracts of all medications due to the presence of reductants, or antioxidants. As a result, the production of Perl's Prussian blue at 700 nm can be used to measure Fe^{2+}. Table 2 and Fig. 1A present the findings of the plant extracts' reductive capacities in relation to ascorbic acid, categorized by concentration. Depending on each drug's reducing power, the test solution's yellow color in this assay changes to different colors of green and blue. Reductones showed reducing characteristics that functioned as antioxidants by dissolving the chain of free radicals and contributing an atom of hydrogen. Ascorbic acid is comparable in its effect to the significantly reduced efficacy of extracts of amla, coriander, amomum, and saffron, depending on their concentration. Sandal and cassia had the least amount of effect, whereas jatamansi and borage exhibited the least amount of lowering efficacy.

DPPH free radical scavenging method:

The technique relies on the reduction of a methanolic DPPH solution in the presence of an antioxidant that donates hydrogen, as a result of the reaction producing the non-radical form DPPH-H. Similar to regular ascorbic acid, the extract proved effective in reducing the stable radical DPPH to the yellow-colored diphenyl picrylhydrazine. Because of its capacity to donate hydrogen, ascorbic acid has been discovered to decrease and decolorize 1, 1-diphenyl-2-picrylhydrazyl. The DPPH free radical was inhibited by the extracts of amla, amomum, coriander, borage, saffron, and jatamansi, which demonstrated a significant concentration-dependent antioxidant activity (IC_{50} values of 19.23, 29.42, 30.02, 31.08, and 42.85 μg mL^{-1}, respectively). Ascorbic acid, a major marker constituent of amla, had an IC_{50} value of 16.41 μg mL^{-1} when used as a standard (Table 3 and Fig. 1B). It was discovered that the amla extract functioned as an antioxidant and had hydrogen-donating properties about equivalent to those of ascorbic acid. As the concentration of the extract and ascorbic acid grew (5 - 25 μg mL^{-1}), so did the scavenging action. Comparing the sandal and cassia extracts to other drugs and ascorbic acid, they had reduced activity (IC50 values of 62.64 and 51.32 μg mL^{-1}).

Nitric oxide scavenging method:

Potent pleiotropic mediator of physiological processes, nitric oxide (NO) inhibits platelet aggregation, regulates cell-mediated toxicity, and acts as a smooth muscle relaxant. As an effector molecule in various biological systems, such as neuronal messenger, vasodilatation, and antibacterial and anticancer actions, it is a diffusible free radical[18]. Nitric oxide has been linked to inflammation, cancer, and other pathological disorders in addition to reactive oxygen species. The phytochemicals may be able to block the effects of NO creation, which might be very useful in reducing the negative health effects associated with the body's overproduction of NO. Moreover, the scavenging action might aid in stopping the harmful neurodegenerative cycle of events that excess NO production starts. The extracts of amla, amomum, saffron, borage, coriander, jatamansi, and saffron showed significant dose-dependent activity between 10 and 50 μg mL^{-1} with IC_{50} values of 32.6, 54.55, 52.57, 56.96, 63.49, and 71.71 μg mL^{-1} (Table 4 and Fig. 1C), while ascorbic acid's IC50 value was 15.94 μg mL^{-1}. The nitric oxide free radical is least effective against sandal and cassia, with IC_{50} values of 187.18 and 215.46 μg mL^{-1}, respectively.

Table 1: Total phenolic contents in all drug samples

Drug	**Phenolics** % w/w (Mean ± SD*)
Amla	20.82 ± 1.60
Coriander	2.21 ± 0.03
Borage	1.28 ± 0.07
Cassia	1.04 ± 0.02
Amomum	2.74 ± 0.18
Jatamansi	4.12 ± 0.09
Saffron	3.49 ± 0.13
Sandal	0.96 ± 0.06

*Standard Deviation

Table 2: The absorbance showing reducing power of all samples at different concentrations

Conc.	**Ascorbic acid**	**Amla**	**Jatamansi**	**Borage**	**Coriander**	**Sandal**	**Amomum**	**Saffron**	**Cassia**
	Mean Absorbance ± RSD*								
20	0.275 ± 0.005	0.173 ± 0.0021	0.167 ± 0.0051	0.182 ± 0.0040	0.166 ± 0.0042	0.137 ± 0.0051	0.098 ± 0.0046	0.123 ± 0.0021	0.084 ± 0.0031
40	0.386 ± 0.010	0.273 ± 0.0051	0.198 ± 0.0040	0.250 ± 0.0059	0.193 ± 0.0021	0.158 ± 0.0026	0.151 ± 0.0042	0.175 ± 0.0049	0.099 ± 0.0050
50	0.459 ± 0.009	0.332 ± 0.0051	0.232 ± 0.0038	0.291 ± 0.0038	0.287 ± 0.0040	0.188 ± 0.0032	0.194 ± 0.0031	0.278 ± 0.0064	0.147 ± 0.0044
100	0.683 ± 0.006	0.495 ± 0.0036	0.309 ± 0.0021	0.432 ± 0.0060	0.467 ± 0.0078	0.242 ± 0.0053	0.383 ± 0.0050	0.461 ± 0.0026	0.205 ± 0.0021
200	1.189 ± 0.012	0.777 ± 0.0049	0.523 ± 0.0021	0.573 ± 0.0042	0.691 ± 0.0065	0.381 ± 0.0050	0.687 ± 0.0056	0.643 ± 0.0040	0.281 ± 0.0031

*Relative Standard Deviation

Table 3: The results of % inhibition showing DPPH radical scavenging efficacy of all samples at different concentrations with respective IC_{50} values

Conc.	Ascorbic acid	Amla	Jatamansi	Borage	Coriander	Sandal	Amomum	Saffron	Cassia
	% Inhibition ± Standard Deviation (SD*)								
5	14.43 ± 1.05	10.58 ± 0.64	11.35 ± 0.15	5.10 ± 0.06	4.32 ± 0.19	4.57 ± 0.21	13.76 ± 0.17	11.38 ± 0.10	7.77 ± 0.90
10	25.02 ± 0.72	13.50 ± 0.80	15.34 ± 0.40	12.37 ± 0.68	11.03 ± 0.16	7.44 ± 0.19	19.96 ± 0.45	15.39 ± 0.34	11.56 ± 0.49
15	41.87 ± 0.61	25.00 ± 0.44	18.95 ± 0.51	19.64 ± 0.31	12.11 ± 0.45	11.10 ± 0.33	27.22 ± 0.58	22.13 ± 0.49	16.12 ± 0.58
20	65.92 ± 1.57	41.64 ± 0.65	25.47 ± 0.34	30.98 ± 0.39	29.41 ± 0.31	15.03 ± 0.30	35.91 ± 0.38	29.49 ± 0.49	20.64 ± 0.29
25	78.82 ± 0.88	55.71 ± 0.47	32.44 ± 0.66	39.97 ± 0.70	42.34 ± 0.28	20.83 ± 0.62	43.75 ± 0.46	37.82 ± 0.43	26.32 ± 0.23
IC_{50}	**16.41**	**19.23**	**42.85**	**31.08**	**30.02**	**62.64**	**29.42**	**34.99**	**51.32**

*Standard Deviation

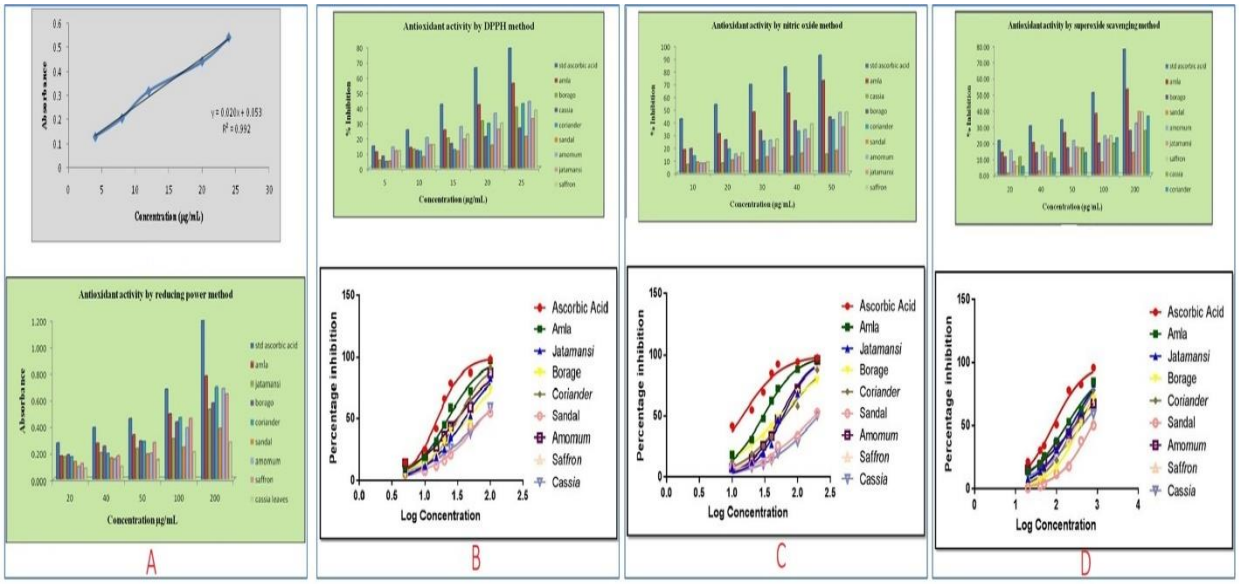

Figure 1: Calibration plot for total phenolic contents and comparative antioxidant efficacy of all samples by reducing power method (A), Comparative antioxidant efficacy of all samples and comparative dose response curve between percent inhibitions against log concentration by DPPH method (B), Nitric oxide method (C), and Superoxide anion scavenging method (D)

Table 4: The results of % inhibition showing nitric oxide radical scavenging efficacy of all samples at different concentrations with respective IC_{50} values

Conc.	Ascorbic acid	Amla	Jatamansi	Borage	Coriander	Sandal	Amomum	Saffron	Cassia
	% Inhibition ± Standard Deviation (SD*)								
10	42.40 ± 0.96	18.15 ± 0.33	7.58 ± 0.20	19.06 ± 0.48	13.21 ± 0.29	8.41 ± 0.33	7.60 ± 0.26	8.48 ± 0.29	6.53 ± 0.17
20	53.69 ± 0.17	30.73 ± 0.59	12.30 ± 0.30	25.84 ± 0.56	18.64 ± 0.08	9.93 ± 0.09	14.60 ± 0.34	15.50 ± 0.45	7.65 ± 0.23
30	69.47 ± 0.65	47.76 ± 0.49	19.60 ± 0.50	33.14 ± 0.46	24.92 ± 0.24	12.49 ± 0.22	25.51 ± 0.29	26.30 ± 0.29	9.93 ± 0.27
40	83.18 ± 0.35	62.53 ± 0.54	26.60 ± 0.46	40.92 ± 0.76	32.79 ± 0.63	15.40 ± 0.17	33.83 ± 0.25	38.43 ± 0.19	12.93 ± 0.18
50	92.45 ± 0.29	72.58 ± 0.45	35.97 ± 0.69	43.87 ± 0.25	41.67 ± 0.87	17.53 ± 0.29	47.34 ± 0.60	47.48 ± 0.37	14.64 ± 0.16
IC_{50}	**15.94**	**32.61**	**71.71**	**56.96**	**63.49**	**187.18**	**54.55**	**52.57**	**215.46**

*Standard Deviation

Table 5: The results of % inhibition showing superoxide anion scavenging efficacy of all samples at different concentrations

Conc.	Ascorbic acid	Amla	Jatamansi	Borage	Coriander	Sandal	Amomum	Saffron	Cassia
	% Inhibition ± Standard Deviation (SD*)								
20	21.37 ± 0.27	13.80 ± 0.20	7.98 ± 0.25	4.90 ± 0.23	5.24 ± 0.09	0.58 ± 0.07	15.18 ± 0.07	5.38 ± 0.05	10.94 ± 0.16
40	30.47 ± 0.22	19.90 ± 0.57	13.90 ± 0.26	7.18 ± 0.20	9.90 ± 0.09	2.32 ± 0.06	18.35 ± 0.06	10.80 ± 0.11	13.53 ± 0.14
50	34.19 ± 0.16	25.88 ± 0.27	17.11 ± 0.23	9.60 ± 0.81	13.51 ± 0.12	4.12 ± 0.11	21.31 ± 0.22	16.43 ± 0.08	16.53 ± 0.16
100	50.79 ± 0.16	37.73 ± 0.13	21.85 ± 0.09	15.70 ± 0.11	22.70 ± 0.18	7.70 ± 0.15	24.16 ± 0.11	24.20 ± 0.12	19.43 ± 0.18
200	78.01 ± 0.27	52.99 ± 0.36	39.11 ± 0.16	32.69 ± 0.89	36.31 ± 0.10	13.64 ± 0.14	31.78 ± 0.07	38.71 ± 0.14	27.47 ± 0.11
IC_{50}	**105.08**	**176.63**	**259.25**	**315.98**	**275.58**	**702.08**	**275.19**	**257.90**	**454.60**

*Standard Deviation

Superoxide anion radical scavenging activity:

It is commonly known that during aging and pathological events such ischemia reperfusion injury, superoxide anions generate H_2O_2, -OH, peroxy nitrite, or singlet oxygen, which can cause direct or indirect damage to biomolecules. Lipid peroxidation has also been seen to be directly triggered by superoxide[19]. The PMS-NADH method was used to measure the superoxide anion radical scavenging activity of the methanolic extracts of all medications.

As the quantities of the medicines increased, so did their ability to scavenge superoxide. Table 5 and Figures 1D compare the antioxidant activity of the extract with ascorbic acid. The methanolic extracts of amla, saffron, jatamansi, amomum, and coriander were reported to have half inhibitory concentrations (IC_{50}) of 176.63, 257.9, 259.25, 275.19, and 275.58µg mL^{-1}, respectively, while ascorbic acid's IC50 value was 16.15µg mL^{-1}. Sandal and cassia showed the least amount of action, with IC_{50} values of 702.08 and 454.6µg mL^{-1}, respectively.

Correlation Between Different *In-vitro* Antioxidant Methods:

Figure 2A–2B show the findings of the various *in-vitro* methods used to scavenge free radicals and compare their efficacy for all samples. The presence of phenolic components and the reduction potential are the primary factors contributing to the antioxidant activity of herbs. Each drug's ability to scavenge radicals and reduce their overall phenolic content was also evaluated, and the results are shown in Fig. 2C–2D.

DISCUSSION:

The formulations used in Ayurveda, Unani and other traditional medicine systems mainly prepared in water or sometimes prepared by fermentation process. It showed that the main activity of drugs is in aqueous and alcoholic soluble fractions. As a result, aqueous methanolic extracts of all drug samples have been used in in-vitro antioxidant investigations as a bioactivity-guiding method to choose the right drug sampled for additional in-vivo cardioprotective activity in rodent model. A growing body of research indicates that free radicals formed from oxygen (ROS) and reactive oxygen species (ROS) may be involved in the development of numerous pathological states and diseases, such as rheumatoid arthritis, diabetes, atherosclerosis, and aging[20-22]. Investigating the potential benefits of natural antioxidants is crucial in order to shield the human body from free radicals, which have the potential to cause chronic illnesses[23-24].

Numerous plant-based antioxidant substances have been found to be either potent oxygen scavengers or free radicals[25]. The search for naturally occurring antioxidants to replace synthetic antioxidants, which are being limited due to adverse effects like carcinogenicity, in foods and pharmaceutical materials has gained significant traction recently[26]. Increased consumption of fruits and vegetables has been linked to a decreased risk of cancer, particularly colorectal cancer (GI cancer)[27-28]. It is now commonly acknowledged that the presence of various antioxidants[29], particularly polyphenols, is what gives fruit and vegetables their ability to protect against disease[30-31].

Thus, the DPPH free radical scavenging, super oxide free radical scavenging, nitric oxide free radical scavenging, and reducing power methods have all been used to evaluate the antioxidant capacity of eight medications. The colorimetric approach was also employed to measure the total phenolic contents[32-33]. The outcomes demonstrated that, in comparison to ascorbic acid, sandal and cassia leaves had the least amount of efficiency against free radicals, while amla and saffron had the most potential. Amla and saffron are both known drugs. Amla was studied for its protective role against isoproterenol-induced cardiotoxicity in rats. Pretreatment with amla showed significant preservation of antioxidants, left ventricular function, hemodynamic and contractile function, and marker enzymes specific to myocyte injury and significant inhibition of lipid

peroxidation. These results demonstrated the drug's potential for cardioprotection, which is explained by its potent antioxidant and free radical scavenging activity[34-35]. The medication was found to have a notable antiradical efficacy when taken alone or in conjunction with vitamin E, C, or A. Using adaptogen, the antioxidant capacity of amla was investigated. The findings indicated that the medication may be useful for protecting proteins from thiols and for decreasing ferric and hydrogen peroxide as well as for singlet oxygen scavenging and ferrous chelating[36-37]. Research on the in vitro antioxidant properties of saffron extract has demonstrated the drug's potential[38]. When ethanolic extracts of saffron, crocin, safranal, and normal saline were given intraperitoneally to rats, they had a protective effect against lower limb ischemia-reperfusion[39]. An aqueous-ethanol extract of saffron was examined for its effects on heart rate and contractility on isolated guinea-pig hearts. The results indicated that the aqueous-ethanol extract of saffron had a strong inhibitory impact on the calcium channel of the guinea-pig heart[40]. It was found that saffron extract's antioxidative function is essential to its therapeutic effects and provides a pathogenetic foundation for its use in the treatment of visual impairments resulting from a variety of causes[41].

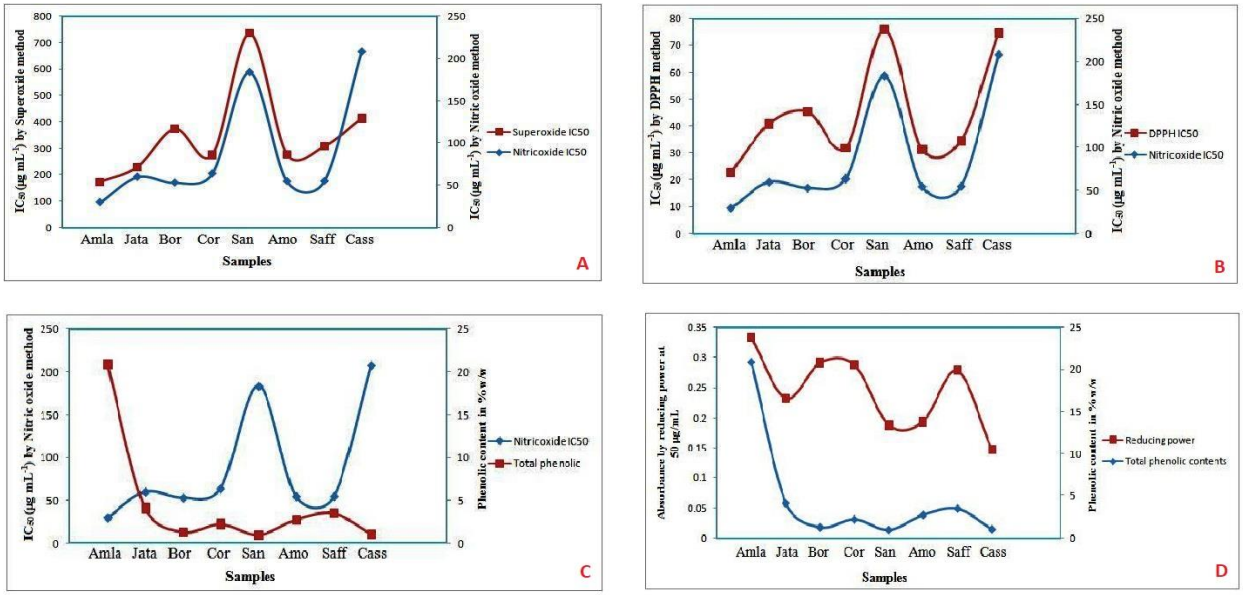

Figure 2: Correlation between nitric oxide free radical scavenging activity with super oxide free radical scavenging efficacy (A), Correlation between nitric oxide free radical scavenging activity with DPPH free radical scavenging efficacy (B), Correlation between total phenolic contents with nitric oxide free radical scavenging efficacy (C), and Correlation between total phenolic contents with reducing power of all drug samples (D).

Amomum, coriander and jatamansi have been showed good protection against free radical and as reducing capacity. These three drugs withdrew interest because coriander and amomum having less reported work as antioxidant and these both spices have not been studied for cardioprotection whereas jatamansi having some reported work for cardioprotective role of the drug. Because of its high polyphenol content, potent reducing power, and superoxide radical scavenging action, amomum has been shown to significantly inhibit LPO. Four cyclic diarylheptanoid components were extracted from amomum and their DPPH radical-scavenging activity was investigated[42-43]. Glutathione levels, lipid conjugated dienes, and the antioxidant effect of amomum on hepatic and cardiac antioxidant enzymes were all investigated in rats given a high-fat diet in addition to the medication[44]. It has been noted that the drug's capacity to activate antioxidant enzymes provides antioxidant protection. Studies using liver histopathology, serum glutamyl oxaloacetic acid transaminase (SGOT), serum glutamyl pyruvate transaminase (SGPT), alkaline phosphatase (ALP), and bilirubin have assessed the hepatoprotective potential of coriander against carbon tetrachloride toxicity in rodent models. By lowering liver weight, mentioning enzyme activity, and directly lowering bilirubin in rats that were drunk with carbon tetrachloride, the medication demonstrated hepatoprotection, which may be related to the antioxidant potential of phenolic compounds[45-46]. The phenolic and flavonoid compounds in coriander have been shown to have some of its high antioxidant activity[47]. Additionally, coriander has been shown to have radical scavenging, reducing power, and lipid per oxidation inhibition activity[48-49]. Coriander seeds have been shown to have both antihyperglycemic and antioxidative properties[50]. Research revealed that coriander extract had a cardio-depressant effect in guinea-pig atria and promoted vasodilatation against

phenylephrine and K+-induced contractions in rabbit aortas. In rats, it also caused diuresis. Coriander's hypotensive impact was also investigated in rats under anesthesia, yielding noteworthy findings[51].

The cardioprotective properties of jatamansi have been investigated through studies on Wistar albino rat's serum and cardiac lipid metabolism in response to doxorubicin-induced myocardial damage. When rats given a seven-day oral jatamansi extract pretreatment, their doxorubicin-induced lipid status was significantly prevented, as demonstrated by the actions of lipid metabolizing enzymes. Additionally, there was a link between the biochemical parameters and histopathological data. According to the findings, jatamansi may have a hypolipidemic and protective effect on rats' hearts after doxorubicin-induced myocardial injury. This effect may be due to the herb's anti-lipid peroxidative qualities[52]. In doxorubicin (adriamycin)-induced cardiac damage, the impact of jatamansi rhizomes on biochemical alterations, tissue peroxidative damage, and aberrant antioxidant levels was measured. While valeranone is a known sesquiterpene isolated from jatamansi that has been pharmacologically investigated in animal experiments for its sedative, tranquilizing, and antihypertensive properties, the pretreatment with jatamansi extract significantly prevented these alterations and restored the enzyme activity and lipid peroxides to near normal levels[53]. Three additional pharmacological models showed evidence of the anti-ulcer activity. Weak hypotensive activity was seen since valeranone's activity was lower than that of the standard compounds used[54].

These drugs have demonstrated strong antioxidant properties. They were originally studied utilizing four techniques, total phenolic contents at the same time, and correlation between all the techniques was found. The medication with higher phenolic content demonstrated greater reducing and free radical scavenging activity, leading to the noteworthy results. The medication with lower phenolic content demonstrated lower reducing and lower free radical scavenging effectiveness. For their potential to protect the heart, jatamansi, coriander, and amomum were also chosen for in-vivo research. Although jatamansi has been shown to protect the heart, its exact mechanism is unknown. In the following investigation, we have examined these medications' mechanistic effects on oxidative stress, elevated inflammatory marker levels, and doxorubicin-induced apoptosis. The study will examine the histological alterations in the cardiac tissues of four groups: control, toxic, standard, and drug-treated. In addition, standard marker DNA will be isolated from each group, and the resulting DNA fragmentation pattern will be examined to provide valuable insight into the toxicity of the DOX group and the protective effects of the medications.

CONCLUSION:

In conclusion, the research work demonstrated that all herbal drugs have differences in their phenolic content. It has been observed that, if a drug has a low amount of phenolic content, it is showing lower value of reducing capability, similarly if a drug has more amount of phenolic content, it is showing a strong reducing power, respectively. In similar pattern, the results of different free radical scavenging potential have been found to complement each other. The drug containing higher phenolic content it is having less IC_{50} value means that it will need less concentration to scavenge the free radicals, respectively. In this way, this methodology can be adopted to screened out the different plants, extracts and isolated fractions to finalize of choose the best possible active drug, extract or isolated fraction, respectively.

DECLARATION OF COMPETING INTEREST:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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