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# ANTIOXIDANT POTENTIAL OF VARIOUS EXTRACTS OF *COCCINA INDICA*, *SIDA CORDATA*, *MEDICAGO SATIVA* AND *WEDELIA TRILOBATA* USING DPPH METHOD

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## ABSTRACT

Background - As an element safeguarding the body's health, antioxidants are crucial. Antioxidants may lower your chance of developing chronic diseases including cancer, Liver and heart disease, according to scientific research. The majority of the antioxidant substances in a regular diet come from plant sources and fall under several groups of substances with a wide range of physical and chemical characteristics.

Objective - To evaluate the Antioxidant activity of the various extracts of *Coccina indica*, *Sida cordata*, *Medicago sativa* and . *Wedelia trilobata* leaves using 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) Method.

Methods –

The selected plant components are crushed into a fine powder, and this powder is then sieved through a specific number of sieve 40 to create a homogenous powder. Additionally, this powder has been tested in accordance with WHO guidelines. It was discovered that all of the measured parameters fell inside the defined ranges. All of the plant parts were extracted using pet ether, chloroform, ethanol, and aqueous after physicochemical evaluation.

Results –

In this study Antioxidant activity was performed by DPPH (1, 1diphenyl-2-picrylhydrazyl) radical scavenging method for different extracts of leaves of of *Coccinaindica*, *Sidacordata*, *Medicago sativa* and *Wedeliatrilobata*. plant species which showed that alcoholic extract of leaves of this plant on higher concentration possess better antioxidant potential when compare to reference standard ascorbic acid. They exhibited strong antioxidant DPPH radical scavenging activity with IC50 value of9.3 and 24.8 µg/ml for

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ascorbic acid and alcoholic leaves extract respectively. The absorbance for reducing power was found to be 0.0390, 0.0989 for ascorbic acid and alcoholic leaves extract respectively. The strongest antioxidant activity of ethanol extract could be due to the presence of flavonoids and phenols.

**Keywords:** DPPH, Antioxidant, *Coccina indica*, *Sida cordata*, *Medicago sativa*, *Wedelia trilobata*

## INTRODUCTION

Several ways for obtaining drug discovery molecules have been utilized, including plant isolation and various herbal sources. Even if novel chemical buildings are now not developed throughout drug improvement from medicinal plants, current compounds with clean organic recreation may additionally supply essential drug outcomes.

An anti-oxidant is a substance that stops oxidation of additional chemicals. They protect the crucial cell by reducing the negative effects of components. Free radicals, which are organic cell waste products, liberation of free radicals during metabolism. Chemical processes in the body that are metabolised or produced species with an outermost unpaired electron (valance) shell of the molecule. This is the cause, or reason Since they are extremely receptive, free radicals can interact with proteins, lipids, carbs and DNA. They are free radicals attack the nearest stable molecules, stealing its electron. When the attacked molecule loses its electron, it becomes a free radical itself, beginning a chain reaction that led to the eventual description of a alive cell. Free radicals may be either oxygen derived derived from (ROS, reactive oxygen species) or nitrogen (RNS, reactive nitrogen species). the oxygen resulting substances are O- superoxide, hydroxyl, and oxygen two ROO[peroxyl], ROO[alkoxyl], and [hydroperoxyl] as free oxygen as a non-radical and H2O2 as a radical. Nitrogen the majority of derived oxidant species are NO [nitric oxide], ONOO [peroxy nitrate], NO2 [nitrogen dioxide] and Dinitrogen trioxide, with DNA, lipids, proteins, and carbohydrates. They target the closest stable molecules by taking their electrons. When the attacked molecule loses its electron, it transforms into a free radical, setting off a series of events that eventually led to the description of a living cell. Free radicals can come from either nitrogen or oxygen (ROS, reactive oxygen species) (RNS, reactive nitrogen species). O-superoxide, hydroxyl, oxygen two, ROO[peroxyl], ROO[alkoxyl], and [hydroperoxyl] as free oxygen as a non-radical are the products of oxygen.3 and the radical H2O2. Nitric oxide (NO), peroxy nitrate (ONOO), nitrogen dioxide (NO2), and dinitrogen trioxide make up the majority of nitrogen's derived oxidant species.or N2O3, is present in normal cells as balanced ratio of antioxidants to oxidants However, When production species are changed, this balance could change. raised or when levels of antioxidants are diminished. Oxidative stress is the term for this phase. The damage caused by oxidative stress to biopolymers including proteins, polyunsaturated fatty acids, and nucleic acids. both acids and sugars. Peroxidation of lipids is oxidative deterioration of polyunsaturated lipids and it involves transition metal ions and ROS. It is a Molecular mechanisms of cell damage that result in a wide range of cytotoxic products, most of which are Malondialdehyde (MDA), for example, 4- HNE, or oxidative stress, results in significant human illnesses caused by cell injury include a wide range. similar to Parkinson's disease, Alzheimer's illness atheroscleorosis, cancer, arthritis, immunological incompetence and neurodegenerative diseases, etc. A lack of nutritional antioxidants also results in oxidative stress, which signifies the identification of natural anti-oxidative agents present in die consumed by human population.

There are various other indigenous plants showing there antioxidant potential thus helps in treating various aliments of the human body other than those mentioned in the ayurvedic texts. *Coccinaindica*, *Sidacordata*, *Medicago sativa*, *Wedeliatrilobata are*investigated for scientific validation of their uses in various human disease so as to validate the antioxidant potential in various extracts.

## MATERIAL AND METHOD

### Plant Material

*Coccinaindica* leaf material was collected from Local Vegetable Market, Indore,Madhya Pradesh. *Sidacordata* leaf material was collected from Herbal garden, Government Agriculture College, Mandsaur, Madhya Pradesh. *Medicago sativa* leaf material was collected from Medicinal Plants Garden, Campus of Acropolis Institute of Pharmaceutical Education & Research, Indore. *Wedeliatrilobata* leaf material was collected from Medicinal Plants Garden, Campus of Acropolis Institute of Pharmaceutical Education & Research, Indore

Plant material (leaves) selected for the study were washed thoroughly under running tap water and then rinsed in distilled water; they were allowed to dry for some time at room temperature. Then the plant material was shade dried without contamination for about 3 to 4 weeks.

### Preparation of Plant Extract

The powdered plant materials were placed in a 5 liter conical flask with various solvents like $CHCl_3$, Ethanol, Pet.ether and water distilled for imbibing. The Maceration method of extraction was used for the extraction. The vessel is sealed and held for seven days, with some shaking. The liquids were strained after efficient extraction, and marc was pressed, adding the expressed liquid to the strained liquids. Decanting or filtering were used to clarify the mixed liquid. The mixer of solution were concentrated an a water bath and then dried out.

### Total Phenolic Content

The total phenolic content of the samples (extracts) was evaluated using Folin reagent. Folin-reagent Ciocalteau's was used to oxidise the extracts, which were then neutralised with sodium carbonate. After 60 minutes, the absorbance of the blue colour solution was measured at 765nm using gallic acid (GA) as a reference. The samples' total phenolic content was estimated as mg gallic acid equivalents (mg.GAE)/g.

### Procedure

100 mg gallic acid was disolved in 100 ml ethanol to make a gallic acid stock solution (1000g/ml). The stock solution was used to make various dilutions of standard gallic acid. Calibration curve was plotted by mixing 1ml aliquots of 10, 20, 40, 80, 160&320µg/ml of gallic acid solutions with 5.0 ml of Folin-Ciocalteu reagent (0.2N) and 4.0 ml of sodium carbonate solution (75g/l). After 30 minute 20 degree celcius the absorbance was measured at 765nm, saperately, 1 ml of the sample /extract (1mg/ml) was mixed, with the same chemicals used in the standard curve creations., and the absorbance was measured after 1 hour to determine the total phenolic component in the extract using formula. All experiments were performed in triplicate.

$$C = C_1 \times \frac{V}{M}$$

Where; C = Total phenolic content in mg. GAE/gm; $C_1$= gallic acid concentration from standard curve in mg/ml; V = The volume of extract in ml; M = plant extract weight in gms (g).

The methanolic solution of alpha, alpha-diphenyl-β-picrylhydrazyl (DPPH) was used to test extracts for free radical scavenging activity. DPPH reacts with antioxidants and get converted to hydrazine alpha, alpha-diphenyl-β-picryl (Fig. 11). The amount of discolouration shows how effective the antioxidant extract is scavenging free radical. Antioxidant activity has been measured by the change in absorbance produced at 517nm. The sample volume necessary to achieve a 50% drop in the original DPPH

concentration is known as the IC50 value. The sample volume necessary to achieve a 50% drop in the original DPPH concentration is known as the IC50 value.

**Preparation of stock solutions of extracts:**

With analytical grade ethanol, extracts and standard stock solutions were prepared and further test solutions of different concentrations were prepared as described below. With ethanol, all solutions were prepared.

A total of 1 ml of 0.3 mMAt room temperature, 2.5 ml of DPPH solution was added to 2.5 ml of sample/standard solution with varying test doses. The With varying test doses, 492.5 ml of DPPH solution was added to 2.5 ml of sample/standard solution. The absorption values at 517nm were calculated after 30 minutes and translated to a percentage of antioxidant activity using the equation below.

$$\% \text{ Anti radical Activity (I)} = \frac{\text{Control Abs} - \text{Sample Abs}}{\text{Control Abs}} \times 100$$

The blank solution was ethanol (1 ml) plus plant extract solution (2.5 ml), while the negative control was DPPH solution The blank solution contained ethanol (1 ml) and a plant extract solution (2. 5 ml), while the DPPH solution served as the negative control. plus methanol. DPPH solution plus each millilitre of regular water served as positive controls (Ascorbic acid). Linear plot regression was used to calculate the IC50 values, denotes the concentration of extracts/test standard and the ordinatere presents middling of three triplicate scavenging power percentages. Each triplicate were performed and I.C. 50 were determined.

## RESULT AND DISCUSSION

The Ethanolic extract of *Cocciniaindica* (CIEE) were found to have higher content of phenolic compounds (113.12±1.74), and also the higher antioxidant activity as per the DPPH assay ($IC_{50}$ = 37.58±1.16).

The Aqueous extract of *Sidacordata* (SCAE) was havingincreased volumes of phenolic contentwas shown to have significant amounts of phenol. (82.18±1.38), However, the Petroleum extract of *Sidacordata* (SCPE) in DPPH assay showed a better antioxidant potential. ($IC_{50}$ = 79.20±1.36).

The Ethanolic extract of *Medicago sativa*(MSEE) was havingincreased volumes of phenolic content was shown to have significant amounts of phenol**.**(37.24 ± 1.32), and the result of DPPH assay shows a potential antioxidant potential ($IC_{50}$ = 112.76±1.38).

The *Wedeliatrilobata* (WTEE) ethanolic extract was found to have higher phenolic compounds content (38.46 ± 1.21), and as per DPPH assay found to have the antioxidant patential.($IC_{50}$ = 118.65±1.27).

A direct correlation between total phenolic contents and antioxidant activity, in all exracts (except *Sidacordata*), were incontestible by analysis done with linear regression. There is a strong link between total polyphenol concentration and anti oxidant activity, as indicated in the results of Cocciniaindica($r^2$ = 0.921); as well as *Medicago sativa*($r^2$ = 0.927) extracts and Wedeliatrilobata ($r^2$ = 0.817). The finding revealed that the antioxidant properties of these extracts are primarily due to polyphenols included in them.

Ubiquitous bioactive assemblages and a diverse crew of secondary metabolites are phenolic compounds that universally occur in higher plants.The quantity of phenolic components in an extract can be roughly estimated using the Folin-Ciocalteu phenol reagent.Though, thethe assay has been said to be nonspecific not only to polyphenols, as stated by a number of researchers. This could also explain why overall polyphenol content and antioxidant activity of Sidacordatahave such a low correlation ($r^2$ = 0.556). Non phenolic compounds play a role in antioxidant activity in the species, according to certain theories.

**Cocciniaindica extracts with Total Phenolic Contents and DPPH Radical Scavenging Activity**

| Drug | Extract | Total Phenolic Contents (mg.GAE/g) | $IC_{50}$ value (µg/ml) | Correlation ($r^2$) |
|---|---|---|---|---|
| ***Cocciniaindica*** | Petroleum ether | 101.32±2.35 | 45.43±1.48 | 0.921 |
| | Chloroform | 56.75±1.12 | 128.45±1.57 | |
| | Ethanol | 113.12±1.74 | 37.58±1.16 | |
| | Aqueous | 24.82±0.68 | 290.54±2.46 | |
| Ascorbic acid | -- | -- | 3.45 ± 0.054 | |

*Values are in Mean±SD, where as n=3, Ascorbic acid: positive reference DPPH.

***Sidacordata*Total Phenolic Contents and DPPH radical activity for scavenging**

| Drug | Extract | Total Phenolic Contents (mg.GAE/g) | $IC_{50}$ value (µg/ml) | Correlation ($r^2$) |
|---|---|---|---|---|
| ***Sidacordata*** | Petroleum ether | 15.21±0.85 | 79.20±1.36 | 0.556 |
| | Chloroform | 36.56±1.04 | 165.31±1.87 | |
| | Ethanol | 24.19±0.75 | 107.78±2.15 | |
| | Aqueous | 82.18±1.38 | 190.11±1.12 | |
| Ascorbic acid | -- | -- | 3.45 ± 0.054 | |

*Values are in Mean±SD, where as n=3, Ascorbic acid: positive reference DPPH.

***Medicago sativa*-Total Phenolic Contents and DPPH radical activity for scavenging**

| Drug | Extract | Total Phenolic Contents (mg.GAE/g) | $IC_{50}$ value (µg/ml) | Correlation ($r^2$) |
|---|---|---|---|---|
| ***Medicago sativa*** | Petroleum ether | 16.51±0.32 | 300.56±1.45 | 0.927 |
| | Chloroform | 23.77±.065 | 213.45±1.83 | |
| | Ethanol | 37.24±1.32 | 112.76±1.38 | |
| | Aqueous | 34.47±1.63 | 174.41±1.69 | |
| Ascorbic acid | -- | -- | 3.45 ± 0.054 | |

*Values are in Mean±SD, where as n=3, Ascorbic acid: positive reference DPPH.

**Total Phenolic Contents and DPPH radical scavenging activity of *Wedeliatrilobata*extratct**

| Drug | Extract | Total Phenolic Contents (mg.GAE/g) | $IC_{50}$ value (µg/ml) | Correlation ($r^2$) |
|---|---|---|---|---|
| ***Wedeliatrilobata*** | Petroleum ether | 14.42±0.41 | 290.65±1.54 | 0.817 |
| | Chloroform | 28.77±.056 | 224.54±1.87 | |
| | Ethanol | 38.46±1.21 | 118.65±1.27 | |
| | Aqueous | 32.47±1.41 | 165.31±1.78 | |
| Ascorbic acid | -- | -- | 3.45 ± 0.054 | |

*Values are in Mean±SD, where as n=3, Ascorbic acid: positive reference DPPH.

## CONCLUSION

The source of rakthavaha and ranjakapitta is the liver (Vakrit) in Ayurveda. As per ayurveda the main factor for the development of any disease in the body is damage to the liver which inturn damage the digestion. Many of the drugs are available as curing the hepatic disorders in the various texts of the ayurveda. There are various other indigenous plants that are used to treat the liver problems other than those mentioned in the ayurvedic texts.

The present study clearly indicated that the ethanolic extracts of the leaves of *the Coccinaindica, Medicagosativa, Wedeliatrilobata and aqueous extract of Sidacordata*had shown a significant Antioxidant potential that can be utilized to treat many harmful diseases.

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