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Exploring Antioxidant Potential Of *Crataeva Nurvala* Leaves: A Comprehensive Pharmacognostic And Physicochemical Insight

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Abstract:
Crataeva nurvala, a member of the Capparidaceae family, commonly known as Varuna, is a medium-sized, deciduous tree that is native to India and other parts of South Asia. Reaching heights of 5-10 meters, it features distinctive trifoliate leaves and fragrant white to yellow flowers that attract pollinators. Traditionally, various parts of *Crataeva nurvala* have been used in folk medicine for their potential health benefits, including metabolic disorders, anti-inflammatory and digestive properties, making it both ecologically and culturally significant. The present research includes a comprehensive pharmacognostic study, detailing the macroscopic and microscopic characteristics of the plant, which aids in its identification and authentication. Physicochemical analysis was also performed to determine moisture content, ash value, and extractive values, which are crucial for ensuring quality control and standardization. Antioxidant assays, including 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Ferric Reducing Antioxidant Power (FRAP), Nitric Oxide (NO), and Superoxide Radical Scavenging Activity (SRSA), were used to evaluate the plant's free radical scavenging potential. Microscopic and fluorescence analyses further support quality control efforts. Ongoing research explores the therapeutic potential and safety of *Crataeva nurvala*. Different antioxidant activity assay methods, revealed that the leaf's aqueous extract ACN has a better IC50 value than ECN in the DPPH, FRAP, and NO assays, indicating stronger antioxidant activity in these tests. ECN only outperforms ACN in the SRSA assay, but the difference is not substantial. ACN shows overall stronger antioxidant activity compared to ECN when considering all four assays. Therefore, ACN is the sample that shows the best antioxidant result compared to the standard (STD) across these assays. This comprehensive evaluation highlights the medicinal value of *Crataeva nurvala*, particularly in its antioxidant properties.

Key words: Quality control, Extract, Phytochemical screening, Assay, Antioxidant activity.

INTRODUCTION:

India's healthcare landscape is characterized by a strong emphasis on traditional herbal medicine, with Ayurveda, Yoga, Unani, Siddha, Homeopathy, and Naturopathy serving the healthcare needs of over 998 million citizens – roughly 70% of the population. This widespread adoption highlights the enduring relevance of herbal-based healthcare systems. Plants have formed one of the sophisticated traditional medicine systems and have been in existence for thousands of years, dating back to early humans. They constitute an effective source of traditional and modern medicines and play an important role in health care programs. [1, 2] The pharmaceutical industry is increasingly turning to plant-derived drugs because of their significant therapeutic potential, diverse chemical profiles, and long-established traditional uses. With growing consumer interest in natural and complementary therapies, the industry is actively incorporating plant-based compounds, utilizing both traditional knowledge and modern technology to ensure efficacy and sustainability. In contemporary medicine, plants serve as sources of direct therapeutic agents, models for developing new synthetic compounds, and taxonomic markers for the creation of more complex semi-synthetic chemical compounds. *Crataeva nurvala*, also known as Varun plant, is a medicinal plant found in India, including Rajasthan, and is used in Ayurvedic medicine and folk medicine. It's a member of the Capparidaceae family. The plant's flowers flowers in March and fruits in June. [3] Authentication and standardization are prerequisite steps, especially for herbal drugs and their formulations in traditional systems of medicine. The present study is focused on the pharmacognostic standardization parametrics such as organoleptic, microscopic, and macroscopic analyses, along with the determination of ash and moisture content, extractive values, foreign matter, and fluorescence characteristics of the leaf of *Crataeva nurvala* as described in the World Health Organization guidelines. [4]

The basic objective of this work was to study pharmacognostic, physicochemical, phytochemical screening and antioxidant potential of *Crataeva nurvala* leaves. Pharmacognostic screening helps in the accurate identification and authentication of medicinal plants. It includes the study of morphological and microscopic characteristics, which are essential to distinguish between closely related species and to ensure the correct plant material is used. Physicochemical screening evaluates parameters such as moisture content, ash value, extractive value, and pH. These parameters are essential for the quality control of herbal medicines, ensuring they meet pharmacopeial standards. Phytochemical

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screening identifies the presence of bioactive compounds such as alkaloids, flavonoids, tannins, saponins, and phenolic compounds. These compounds are often responsible for the therapeutic effects of the plant. [5,6,7,8,9]
The next objective of this study was to evaluate the antioxidant potential using four different antioxidant assay methods: 2,2-diphenyl-1-picrylhydrazyl assay (DPPH), Ferric Reducing Antioxidant Power (FRAP) assay, the Nitric Oxide (NO) antioxidant assay, and the Superoxide Radical Scavenging Activity (SRSA) assay.

MATERIALS & METHODS

Plant Material

Leaves of *Crataeva nurvala* were collected from Smriti Van, Jaipur. The leaves were identified, authenticated and certified by Dr Bharti Vijay, botanist from the Department of Botany, Apex University, Jaipur (Rajasthan).

Organoleptic evaluations

The collected sample of leaves of *Crataeva nurvala* were studied organoleptically, with naked eye and magnifying lens i.e. appearance included size, shape, form, surface features, colour, odour and taste of raw leaf part. The findings were recorded.

Microscopic analysis

Microscopic analysis of the plant was carried out according to the method of Trease and Evans [9]. For microscopic studies, free-hand sections of the leaves were taken and stained with safranin. Photomicrographs were taken by attaching a camera on to the vertical tube of the microscope's head.

Physicochemical analysis

The leaves were shade dried and powdered using a mechanical grinder for powder analysis. The physicochemical characteristics of powdered leaves were determined as per the WHO guidelines [10, 43].

Physico-chemical parameters:

1. Foreign matter

5-10 g of sample was weighted (A1). The sample was spread as thin layer on dish or tray. Then, it was examined for foreign matter in daylight with unaided eye followed by 6X lens and 10X lens in daylight. The remaining sample was passed through a sieve no. 250 to remove dust (Mineral admixture). The remaining sample material was weighted after above process (A2). The percentage of foreign matter was calculated. [11]
% foreign matter = (A1-A2) / (A1) ×100
Where:
A1 = Weight before investigation
A2 = Weight after investigation

2. Determination of loss on drying (LOD)

5 g of powdered sample was weighed and kept in oven at 105ºC for 5 hours (A1). Drying and weighing was continued at half an hour interval until difference between two successive weights were not more than 0.25 percentages (A2). The percentage of loss on drying was calculated.. [11] % moisture content = (Initial weight-Final weight) / (Initial weight of dried plant material) ×100
Where:
Initial weight=Weight before oven drying
Final weight=Weight after oven drying

3. Determination of total ash

2 g of the powdered drug was weighed in a tared silica crucible and incinerated at temperature not exceeding 450°C in muffle furnace. The crucible was cooled and weighed. The procedure was repeated until the constant weights. [11] The percentage of the total ash was calculated using the following formula: % Total ash = (Total ash) / (Initial weight of dried plant material) × 100

4. Determination of acid insoluble ash

The total ash was boiled with 25 ml of 2M hydrochloric acid for 5 minutes and the insoluble matter was collected in a Gooch crucible or on an ashless filter paper, washed with hot water, ignited for 15 minutes at a temperature not exceeding 450°C using a muffle furnace. It was then cooled in a desiccator and weighed. The percentage of acid-insoluble ash was calculated. [11] % acid insoluble ash = (Acid insoluble ash) / (Initial weight of dried plant material) × 100

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5. Determination of water soluble ash

The total ash was boiled for 5 minutes with 25 mL of water. The insoluble matter was collected in a Gooch's Crucible or on an ash less filter paper, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450°C using a muffle furnace. It was subtracted from the weight of the ash to obtain weight of water soluble ash. The percentage of water soluble ash was calculated. [11]

% water soluble ash = (Water soluble ash) / (Initial weight of dried plant material) × 100

6. Determination of alcohol soluble extractive (By cold extraction)

5 g coarsely powdered air-dried drug was macerated with 100 mL of 95% of a ethanol in a closed flask for twenty-four hours. It was then continuously shaken for six hours using rotary shaker and then allowed to stand for eighteen hours. The content was filtered using whatman filter paper 01 (11µm). The filtrate was transferred to a pre-weighed flat bottomed dish and evaporated to dryness on a water bath. Then the dish was kept in oven at 105ºC, and dried to constant weight. The percentage of alcohol-soluble extractive was calculated. % extractive value = (Weight of extract×4) / (Initial weight of air dried drug) × 100

7. Determination of water soluble extractive

Procedure was same as that of alcohol soluble extractive value but distilled water instead of alcohol was used.

8. Determination of ether soluble extractive (By Hot extraction)

Three portions of powder sample was extracted simultaneously on 3 Soxhlet apparatus and briefly each. 20-25 g sample was taken in extraction thimble. It was extracted with 150 ml solvent ether (Petroleum ether, Boiling point 40°C to 60°C) in a continuous extraction apparatus using Soxhlet apparatus for 6 hours, until extractor and thistle tube had colourless solvent. The extract was collected in a pre-weighed petri dish. The extract was evaporated on a water bath. It was dried on a hot air oven at 105°C upto constant weight. The percentage of ether soluble extractive was calculated. [11] % extractive value = (Weight of extract×4) / (Initial weight of air dried drug) × 100

9. Determination of pH value

Concentration of Hydrogen ion concentration was determined by using pH meter of Eutech company (model EU-101). The pH meter was calibrated using 4.0, 7.0 and 9.2 pH chemical buffer standards. 1 g powdered sample of each plant material was dissolved in double distilled water and pH value of each sample recorded by dipping the electrode in solution. [11]

Microbial contamination

Total bacterial and fungal count

Procedure

Sample Preparation

A sample of 10 g of the each plant material was weighed. 100 ml of buffered sodium chloride-peptone solution (pH 7.0) was added.

Preparation of medium

Electronic balance was calibrated; the glasswares and utensil were de-pyrogenated in oven at 250°C for 60 minutes, ingredients of medium were weighed carefully and dissolved in distilled water, shaken well followed by heating. Medium was sterilized in an autoclave at 121°C for 15 min. The ultra-violet light of biosafety cabinet, pass box was switched on for 30 minutes before transferring prepared medium. [11, 43]

10. Total bacterial count

Inoculation, Incubation and Observations

1 mL of test sample was inoculated in 30 mL of soybean casein digest agar media containing petri dish under the biosafety cabinet. The Inoculum was incubated in BOD incubator for 5 days at 35–37°C. Colonies were observed and counted on daily basis during incubation period. After incubation period, average of colonies were calculated. All the experiments were performed in duplicate. The colonies were calculated after completion period of both plates and average colony was calculated. [11,43]

11. Total fungal count

Inoculation, Incubation and Observations

1 mL of test sample was inoculated in 30 mL of sabouraud dextrose agar media containing petri dish under the biosafety cabinet. The Inoculum was incubated in BOD incubator for 7 days at 25°C. Colonies were observed and counted on daily basis during incubation period. After incubation period, average of colonies were calculated. All the

experiments were performed in duplicate. The colonies were calculated after completion period of both plates and average colony was calculated. [11,43]

Fluorescence Analysis
Powdered sample was treated with following chemical reagents and solvents separately. 1N sodium hydroxide in methanol, 1N sodium hydroxide in water, 50% sulphuric acid, 50% nitric acid were used. The fluorescence was observed under day light, short wavelength UV and long wavelength UV light.

Preparation of Leaves Extract
The fresh, undamaged and disease-free leaves were selected and washed thoroughly with sterile double distilled water, shade dried and then coarsely powdered in a blender. The coarse powder was successively solvent extracted in a soxhlet extractor using different solvent (nonpolar to polar) such as petroleum ether, ethyl acetate, ethanol, aqueous (Distilled water). The extracts so obtained were further dried in vacuum desiccators. The residue obtained from the extract was used for further studies by preserving it in refrigerator.

Phytochemical Screening (Qualitative estimation of phytochemicals)
Freshly prepared extracts were tested for the presence of various active phyto-compounds like phenols, tannin, flavonoid, protein, amino acids, reducing sugar, carbohydrates, lipids, saponin, alkaloid, glycosides. They identified by characteristic colour changes and precipitation reactions using standard procedures. [11]

a) Tests for Carbohydrates
Molisch's test
2 ml of 10% solution of powdered plant material was taken in a test tube and 2 ml of Molisch's reagent was added and shaken carefully and then about 1ml. of conc. Sulphuric acid was poured from side of the test tube and allowed to stand for one minute. A purple colour ring at the junction of the two layers, indicates the presence of Carbohydrate.

Benedict's test
4 ml of 10% solution of powdered plant material was taken in a test tube and 1ml of Benedict's solution was added and heated almost to boiling. Formation of green, yellow, orange, red or brown colour in order of increasing concentrations of simple sugar in the test solution, due to formation of cuprous oxide, indicates the presence of reducing sugars.

Barfoed's test
4 ml of 10% solution of powdered plant material was taken in a test tube and heated with 1-2 drop of Barfoed's reagent. Formation of red cuprous oxide within two minutes indicates the presence of monosaccharides.

Fehling solution test
It is generally used for reducing sugars and ketone functional groups. It is composed of two solutions which are mixed in situ. Fehling solution A composed of 0.5% of copper sulphate whereas Fehling solution B composed of Sodium potassium tartarate. Equal volumes of Fehling A and Fehling B solutions were mixed (1 ml each) and 2 ml of aqueous solution of drug was added followed by boiling for 5-10 minutes on water bath. Formation of reddish brown coloured precipitate due to formation of cuprous oxide, indicates presence of reducing sugar.

a) Tests for Alkaloids
Mayer's reagent test
2 ml of 10% solution of powdered plant material was taken in a test tube and 2 ml of the Mayer's reagent (Potassium mercury iodide solution) was added. A white or pale-yellow precipitate, indicates presence of Alkaloids. This test does not used presence of alkaloid with purine group.

Dragendorff's reagent test
2 ml of 10% solution of powdered plant material was taken in a test tube and 2 ml of the Dragondorff's reagent (Mixture of potassium iodide and bismuth sub nitrate solution) was added. An orange precipitate, indicates presence of Alkaloids.

Wagner's test
2 ml sample was taken in a test tube and few drops of Wagner's reagent (dilute iodine solution) was added, formation of reddish-brown precipitate, indicates presence of alkaloids.

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Hager's Test
2 ml of 10% solution of powdered plant material was taken in a test tube and saturated aqueous solution of picric acid was added. An orange yellow precipitate indicates the presence of alkaloids.

b) Test for Amino acids
Ninhydrin test
The Ninhydrin test is used to detect the presence of alpha-amino acids and proteins containing free amino groups. Aqueous test solution when heated with ninhydrin molecules, it gives characteristic deep blue or pale yellow colour due to formation of complex between two ninhydrin molecule and nitrogen of free amino acid.

c) Test for Proteins
Xanthoproteic test
1 ml of 10% aqueous solution of powdered plant material was taken in a test tube and 0.5 ml of conc. nitric acid was added to it. Development of yellow colour indicates the presence of proteins.

Millon's test
The Millon's reagent is a solution of mercuric and mercurous ions in nitric and nitrous acids. 1 ml of 10% aqueous solution of powdered plant material was taken in a test tube and 1-2 drops of Millon's reagent was added. White precipitate was produced, which turned red after heating for 5 minutes on water bath, indicates the presence of proteins with aromatic amino acids.

Biuret test
It is done for the presence of proteins with peptide bonds. 1 ml of 10% aqueous solution of powdered plant material was taken in a test tube and 2 ml of 10% NaOH solution was added followed by few drops of lead acetate solution. It was well heated on water bath for few minutes. Production of black precipitate, indicates presence of sulphur containing amino acids.

Tests for Glycosides
d) Borntragor's test
1 ml of Benzene and 0.5 ml of dilute ammonia solution was added to the 1 ml of 10% aqueous solution of powdered plant material was taken in a test tube. Formation of reddish pink colour indicates, presence of anthroquinone glycosides.
e) Test for Phenolic compound
2 ml of 10% aqueous solution of powdered plant material was taken in a test tube and warmed; to this 2 ml of ferric chloride was added and observed for the formation of green and blue colour, indicates the presence of phenolic compounds.
f)Test for Saponin
Foam test
About 1 ml of aqueous solution of powdered material was diluted with distilled water up to 10 ml and shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of froth, indicates presence of saponin.
g) Test for Steriods
Salkowaski reaction
1 ml of 10% aqueous solution of powdered plant material was taken in a test tube and 2 ml of chloroform, 2 ml of concentrated sulphuric acid was added from the side of test tube. The test tube was shaken for few minutes. Development of red colour, indicates the presence of sterols.
h) Test for Tannins
Ferric chloride test
A 5 % solution of ferric chloride in 90 % alcohol was prepared. Few drops of the solution were added to 2 ml aqueous solution of powdered plant material. Appearance of dark green or deep blue colour, indicates the presence of tannins.

Lead acetate
A 10 % w/v solution of basic lead acetate in distilled water was added to 2 ml aqueous solution of powdered plant material. Development of precipitate, indicates the presence of tannins.

Potassium dichromate
A solution of potassium dichromate was added to 2 ml aqueous solution of powdered plant material. Appearance of dark colour, indicates the presence of tannins.

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i) Test for Flavonoids

1 g of powdered material was dissolved in 5 ml ethanol (95% v/v) and treated with 1-2 drops of concentrated hydrochloric acid, 0.5g of magnesium metal. Appearance of pink, crimson or magenta colour within a minute or two, indicates the presence of flavonoids.

***In-vitro* Anti-oxidant Activity:**

1. DPPH (2,2-Diphenyl-1-picrylhydrazyl) Radical Scavenging Activity

a) Preparation of DPPH reagent

0.1mM solution of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) in methanol was prepared.

b) Preparation of Sample/Standard

Freshly 1 mg/ml methanol solution of extracts of *Crataeva nurvala*/standard was prepared. Different volume of extracts/standard (20 – 100µl) was taken from stock solution in a set of test tubes and methanol was added to make the volume to 1 ml. To this, 2 ml of 0.1mM DPPH reagent was added and mixed thoroughly and absorbance was recorded at 517 nm after 30 minutes incubation in dark at room temperature.

c) Preparation of control

For control, 3 ml of 0.1mM DPPH solution was taken and incubated for 30 min at room temperature in dark condition. Absorbance of the control was taken against methanol (as blank) at 517 nm (Athavale et al., 2012).

Percentage antioxidant activity of sample/standard was calculated by using formula:% Inhibition = [(Ab of control- Ab of sample/ Ab of control x 100] [12 - 18]

2. Ferric Reducing Antioxidant Power (FRAP) assay

Materials Needed:

1. FRAP Reagent:
   - 300 mM acetate buffer (pH 3.6)
   - 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl
   - 20 mM FeCl3·6H2O solution
2. Standard Antioxidant:
   - Typically, ascorbic acid (vitamin C) is used as the standard antioxidant in FRAP assays.
3. Sample Solutions:
   - ECN, ACN (dissolved in appropriate solvents, typically ethanol)
4. Spectrophotometer:
   - To measure absorbance at 593 nm

Procedure:

1. Preparation of FRAP Reagent:
   - Mix 300 mM acetate buffer, 10 mM TPTZ solution, and 20 mM FeCl3·6H2O solution in a ratio of 10:1:1. Prepare fresh before use.
2. Preparation of Standard Curve:
   - Prepare a series of ascorbic acid standards (e.g. 20, 40, 60, 80, 100 µM) by diluting a stock solution of ascorbic acid.
   - Add 0.1 mL of each standard solution to 2.9 mL of FRAP reagent.
   - Incubate the mixture at 37°C for 4 minutes.
   - Measure the absorbance at 593 nm against a blank (FRAP reagent without ascorbic acid).
3. Sample Analysis:
   - Prepare your samples (ECN, ACN) similarly by diluting them if necessary.
   - Add 0.1 mL of each sample to 2.9 mL of FRAP reagent.
   - Incubate the mixture at 37°C for 4 minutes.
   - Measure the absorbance at 593 nm.

Data Analysis:

1. Standard Curve:
   - Plot the absorbance values of the ascorbic acid standards against their concentrations to create a standard curve.
   - Use the standard curve to determine the antioxidant capacity of your samples by interpolating their absorbance values.
2. Calculating Antioxidant Power:
   - The antioxidant power of the samples is expressed as micromoles of ascorbic acid equivalents per gram of sample (µmol AAE/g).

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Notes:

- Prepare fresh FRAP reagent each time to ensure its efficacy.
- The incubation time and temperature should be consistent for all samples and standards.

Formula for the FRAP (Ferric Reducing Antioxidant Power) assay

$$\textbf{FRAP value } (\mu \boldsymbol{M}\textbf{Fe(II) equivalent / g sample}) = \frac{\boldsymbol{\Delta Asample}}{\boldsymbol{\Delta Astandard}} \boldsymbol{X} \frac{\boldsymbol{Cstandard}}{\boldsymbol{Msample}}$$

Here, ΔAsample = The change in absorbance at 593 nm for the sample. This is calculated as:
ΔAsample = Absorbance of the sample – Absorbance of the blank
ΔAstandard = The change in absorbance at 593 nm for the standard solution (like ascorbic acid) this is calculated as:
ΔAstandard = Absorbance of the standard – Absorbance of the blank
Cstandard = The concentration of the standard solution in μM (micromolar)
Msample = The mass of sample in gram (g) [19 - 31]

3. Nitric Oxide (NO) antioxidant Assay

This method is used to evaluate the free radical scavenging activity of compounds against nitric oxide.

Materials

1. Sodium nitroprusside (SNP) solution: Acts as a nitric oxide donor.
2. Griess reagent: Composed of sulfanilamide, phosphoric acid, and N-(1-naphthyl)ethylenediamine dihydrochloride, which reacts with nitrite to form a purple azo dye.
3. Phosphate buffer: pH 7.4.
4. Test samples: The antioxidants being tested. (ECN, ACN)
5. Standard reference: Ascorbic acid

Procedure

1. Preparation of SNP solution: Dissolve sodium nitroprusside in phosphate buffer to a final concentration, typically around 10 mM.
2. Sample preparation: Prepare different concentrations / Different volume of extracts/standard (20 – 100μl) in phosphate buffer.
3. Reaction setup:
 - Mix equal volumes of SNP solution and test sample in a series of test tubes.
 - Incubate the mixture at room temperature for 2-3 hours under light to generate nitric oxide.
4. Griess reagent addition:
 - After incubation, add an equal volume of Griess reagent to each test tube.
 - Allow the reaction to proceed for about 30 minutes at room temperature.
5. Measurement:
 - Measure the absorbance of the resulting solution at 546 nm using a spectrophotometer.
 - A decrease in absorbance indicates higher nitric oxide scavenging activity of the test sample.

Calculation:

Prepare a standard curve using known concentrations of sodium nitrite (NO_2-) to convert absorbance readings into concentrations of nitrite ions.
Calculate the percentage inhibition of nitric oxide radicals using the formula:

$$\text{Percent Inhibition (\%)} = \frac{Acontrol - Asample}{Acontrol} X\ 100$$

Where:
$A_{control}$ = absorbance of control (without the antioxidant sample)
A_{sample} = absorbance of test sample (with the antioxidant)

Interpretation:

- Higher percentage inhibition indicates greater scavenging activity against nitric oxide radicals, suggesting stronger antioxidant potential of the test sample. [32 - 36]

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4. Superoxide radical scavenging activity (SRSA) assay

The superoxide radical scavenging activity assay typically involves the generation of superoxide radicals through a chemical reaction and measuring the sample's ability to inhibit this reaction. The Nitroblue Tetrazolium (NBT) reduction method is one of the common assays used for this purpose. Here's a detailed procedure for conducting this assay:

Materials Required

1. Reagents:
- Nitroblue Tetrazolium (NBT) solution
- Nicotinamide adenine dinucleotide (NADH) solution
- Phenazine methosulfate (PMS) solution
- Phosphate buffer (pH 7.4)
- Sample solutions (different concentrations of the test compound/Plant Extracts)
- Control solution (without sample)

2. Equipment:
- Spectrophotometer
- Microplate reader (optional)
- Pipettes and microcentrifuge tubes
- Incubator or water bath (37°C)

Preparation of Solutions

1. NBT Solution:
- Dissolve NBT in phosphate buffer to make a final concentration of 0.3 mM.

2. NADH Solution:
- Dissolve NADH in phosphate buffer to make a final concentration of 0.936 mM.

3. PMS Solution:
- Dissolve PMS in phosphate buffer to make a final concentration of 0.12 mM.

Assay Procedure

1. Sample Preparation:
- Prepare different concentrations of the test sample (e.g., 100 µg/ml, 80 µg/ml, 60 µg/ml, 40 µg/ml, 20 µg/ml) in phosphate buffer.

2. Preparation of Ascorbic Acid Standard Solutions

A. Stock Solution: Prepare a stock solution of ascorbic acid in phosphate buffer (e.g., 1 mg/ml).

B. Dilution: Prepare different concentrations of ascorbic acid from the stock solution (e.g., 100 µg/ml, 80 µg/ml, 60 µg/ml, 40 µg/ml, 20 µg/ml).

3. Reaction Mixture:
- In a microcentrifuge tube or microplate well, mix the following:
  - 1 ml of NBT solution
  - 1 ml of NADH solution
  - 0.1 ml of the sample solution
  - 0.1 ml of PMS solution
- For the control, use 0.1 ml of phosphate buffer instead of the sample solution.

4. Incubation:
- Incubate the reaction mixture at 25°C for 5 minutes.

5. Measurement:
- After incubation, measure the absorbance at 560 nm using a spectrophotometer.

Calculation of Percent Inhibition

% Inhibition = ((Abs_blank - Abs_sample) / Abs_blank) × 100 [37 - 42]

RESULTS & DISCUSSION

Organoleptic characteristics

The leaf of *Crataeva nurvala* is dark green with a characteristic odour, and slight bitter taste. The organoleptic characteristics of the leaf of plant are summarized in Table 1.

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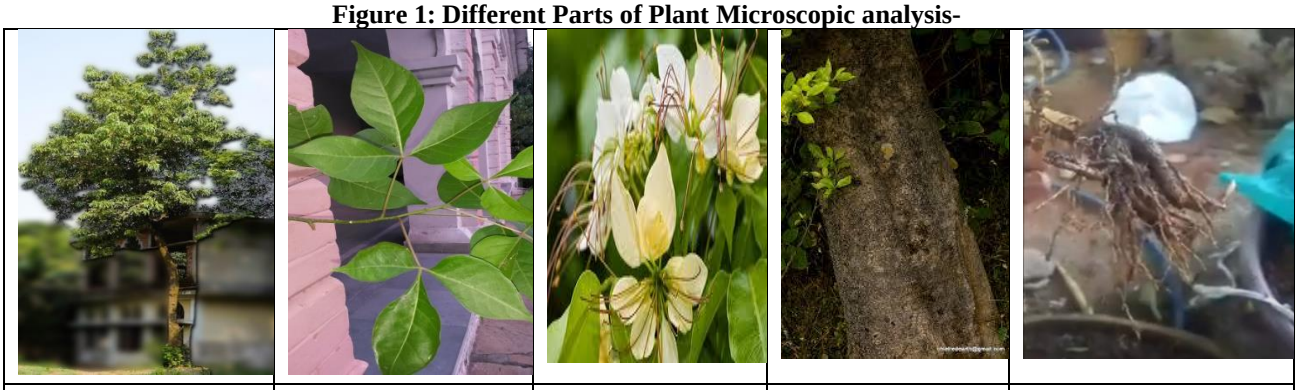
Table 1. Organoleptic characteristics of leaf part:

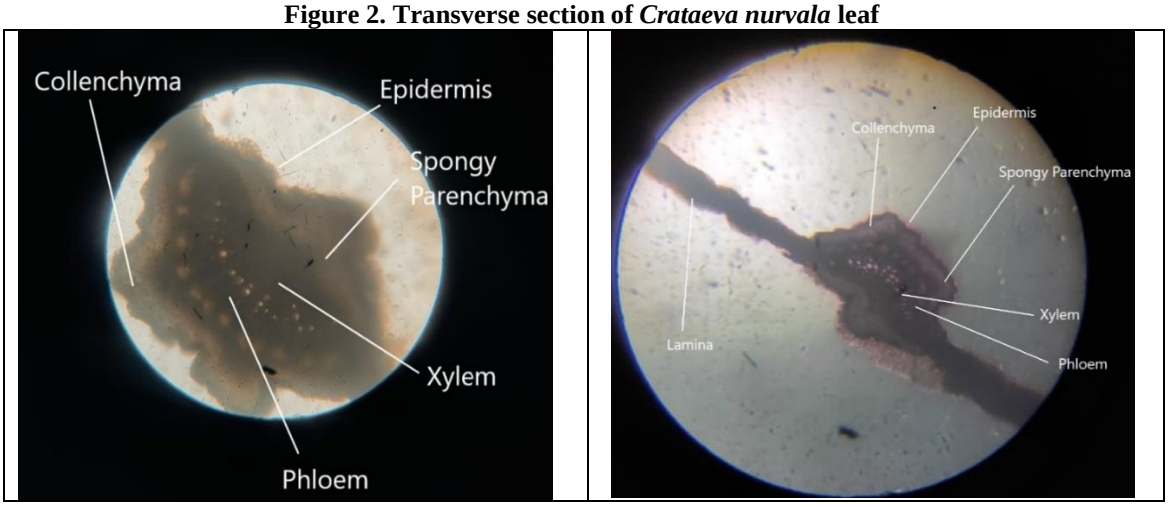
S.No	Organaleptic characteristics	Nature
1.	Appearance	Leaves are trifoliate, with oval leaflets, Tip: Acute or acuminate, Base: Attenuate or rounded, Margin: Entire or slightly serrate
2	Color	Dark Green
3	Odor	Characteristic
4	Taste	Slight Bitter
5	Size	8-15 cm long, 3-6 cm wide
6	Shape	leaflets are ovate, obovate, acute/or acuminate, and reticulately veined

Figure 1: Different Parts of Plant Microscopic analysis-



Whole plant	Leaf	Flower	Stem	Roots

Figure 2. Transverse section of *Crataeva nurvala* leaf



Physio-chemical and microbial analysis (Quality control parameters)

Results of physiochemical and microbial parameters for quality control for each plant material are presented in below mentioned table.

Table 2. Determination of physico-chemical parameters of *Crataeva nurvala* leaves

Sr. No.	Parameter	Results (%w/w)
1.	Foreign matter	0.11
2.	Loss on drying (LOD)	2.68
3.	Total ash	7.54
4.	Acid insoluble ash	0.67
5.	Water soluble ash	1.84
6.	Water soluble extractive value	21.54
7.	Alcohol soluble extractive value	22.32

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Vol 24, No. 4 (2023)
http://www.veterinaria.org
Article received- 02/10/2023 Revised- 09/11/2023 Accepted- 08/12/2023

8.	Ether soluble extractive value	0.88
9.	Determination of pH	5.46
Microbial contamination (cfu/g)		
10.	Total bacterial count	1521
11.	Total fungal count	145

Table 3. Fluorescence behavior of powdered *Crataeva nurvala* leaves treated with different reagents

S. no.	Chemical reagent/solvents	**Observations under UV cabinet**		
		At day light	At short wave length (254nm)	At long wavelength (365 nm)
1.	Powder as such (After rub)	Light Brown	Faint blue	Faint blue
2.	Powder + 1N NaOH in methanol	Yellowish-green	Blue	Green
3.	Powder + 1N NaOH in water	Light green	Blue	Green
4.	Powder + 1N HCl	Light yellow	Faint yellow	Faint orange
5.	Powder + 50% HNO_3	Dark yellow	Yellow	Orange
6.	Powder + 50% H_2SO_4	Light orange	Faint orange	Red

Phytochemical screening

Phytochemical evaluation of various leaves extracts of *Crataeva nurvala* were done for the presence of Alkaloids, Flavonoids, carbohydrate, protein, Saponins, Tannins, Anthraquinones, Phenolic compounds, steroids, Cardiac glycosides, and the result are presented in Table 4.

Table 4. Phytochemical screening of different extract of *Crataeva nurvala* leaves

Name of tests	**Petroleum ether extract**	**Ethyl acetate extract**	**Ethanol extract**	**Aqueous extract**
Carbohydrate				
Molish test	+	-	-	+
Benedict test	-	-	-	-
Fehling test	-	-	+	+
Barfoed test	-	-	+	+
Alkaloids				
Dragendorff test	-	-	+	+
Wagner's test	-	+	+	-
Hager's test	-	-	+	+
Amino acids				
Ninhydrine	-	-	-	+
Protein				
Biuret test	-	-	-	+
Xanthoproteic test	-	-	-	-
Millon test	-	-	-	-
Saponin				
Foam test	-	-	+	+
Glycosides				
Borntrager's test	-	-	-	-
Killer-Killani Test	+	+	+	-
Phenolic compound				
$FeCl_3$ test	-	-	+	+
Flavonoids				
Shinoda test	-	-	++	+
Steroids				
Salkowski test	+	-	+++	-
Tannins				
$FeCl_3$ test	-	-	++	+
Lead acetate test	-	-	+	+
Potassium Dichromate test	-	-	+	+

+++ = Strongly positive, ++ = Moderate Positive, + = Positive, - = Not Detected

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 24, No. 4 (2023)
http://www.veterinaria.org
Article received- 02/10/2023 Revised- 09/11/2023 Accepted- 08/12/2023

As Phenolic compound, Flavonoids and Tannins were present in ethanol and water (polar solvents) and these secondary metabolites were absent in petroleum ether and ethyl acetate (non - polar solvents), ethanolic and aqueous extract of *Crataeva nurvala* were selected for further antioxidant activity study.

Antioxidant activity analysis:

1. DPPH (2,2-Diphenyl-1-picrylhydrazyl) Assay

Table 5 DPPH radical scavenging activity of Ascorbic acid

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.489	49.639
40	0.369	61.997
60	0.282	70.957
80	0.172	82.286
100	0.102	89.495
Control	0.971	
IC50 18.260		

Graph 1 Graph represents the Percentage Inhibition *Vs* Concentration of Ascorbic acid

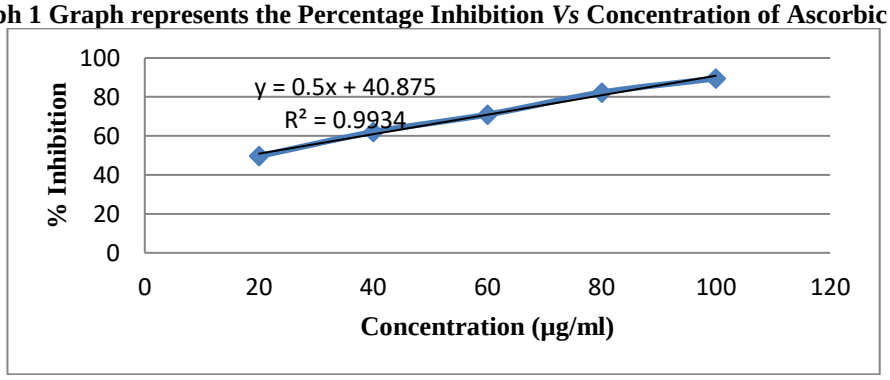
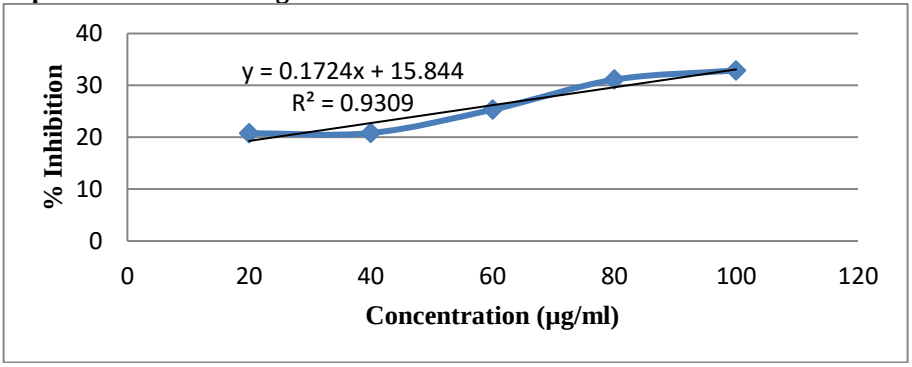


Table 6 DPPH radical scavenging activity of Ethanolic extract of *Crataeva nurvala*

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.769	20.785
40	0.768	20.823
60	0.725	25.334
80	0.669	31.101
100	0.6517	32.883
Control	0.971	
IC50 198.604		

Graph 2 Graph represents the Percentage Inhibition *Vs* Concentration of Ethanolic extract of *Crataeva nurvala*

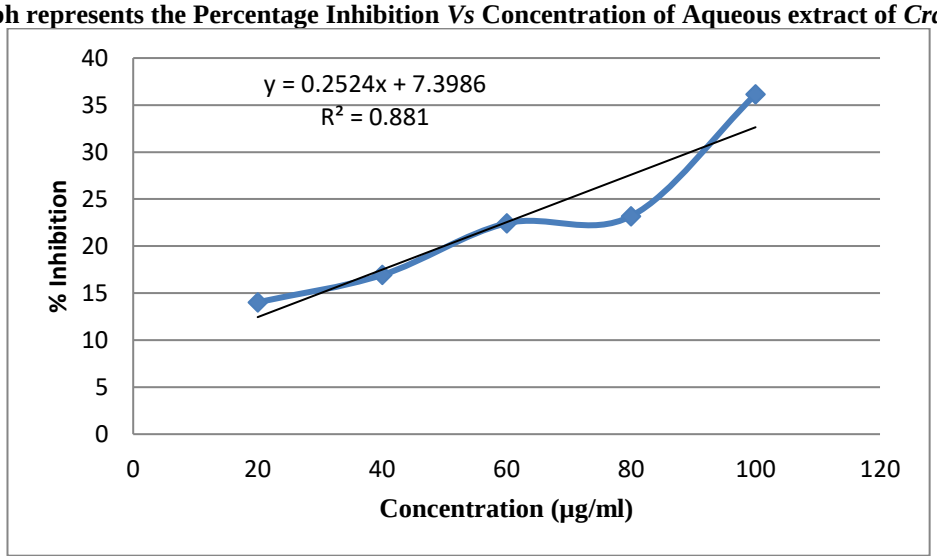


REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 24, No. 4 (2023)
http://www.veterinaria.org
Article received- 02/10/2023 Revised- 09/11/2023 Accepted- 08/12/2023

Table 7 DPPH radical scavenging activity of Aqueous extract of *Crataeva nurvala*

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.834	14.026
40	0.806	16.931
60	0.753	22.430
80	0.745	23.192
100	0.620	36.138
Control	0.971	
IC50 169.055		

Graph 3 Graph represents the Percentage Inhibition *Vs* Concentration of Aqueous extract of *Crataeva nurvala*

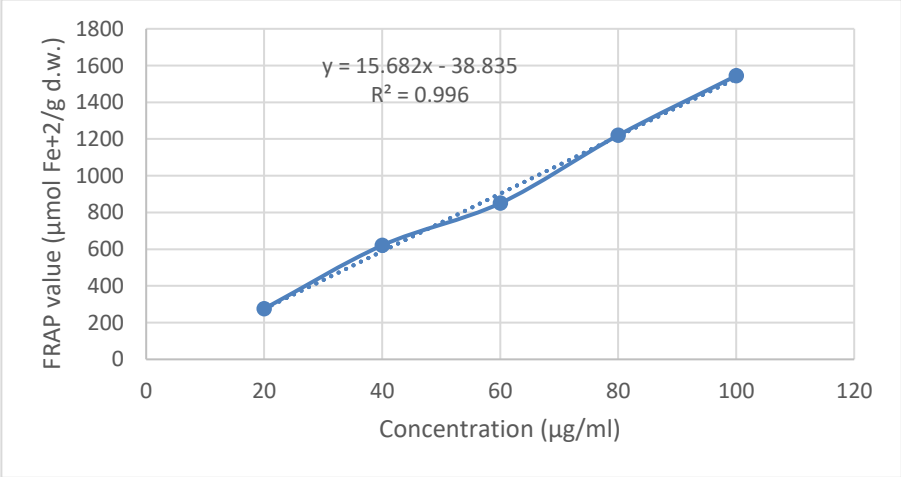


2. Ferric Reducing Antioxidant Power (FRAP) Assay

Table 8 FRAP value of Ascorbic acid

Concentration (µg/ml)	Absorbance (at 593 nm)	FRAP value (µmol Fe+2/g d.w.)
20	0.053	275.54
40	0.241	620.61
60	0.495	850.22
80	0.948	1220.04
100	1.500	1544.03
Control	0.059	
IC50 = 5.66		

Graph 4 Graph represents the FRAP value *Vs* Concentration of Ascorbic acid



REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 24, No. 4 (2023)
http://www.veterinaria.org
Article received- 02/10/2023 Revised- 09/11/2023 Accepted- 08/12/2023

Table 9 FRAP assay of Ethanolic extract of *Crataeva nurvala*

Concentration (µg/ml)	Absorbance (at 593 nm)	FRAP value (µmol Fe+2/g d.w.)
20	0.081	120.42
40	0.151	210.11
60	0.232	350.26
80	0.259	495.55
100	0.388	592.10
Control	0.059	
IC50 = 10.57		

Graph 5 Graph represents the Percentage Inhibition *Vs* Concentration of Ethanolic extract of *Crataeva nurvala*

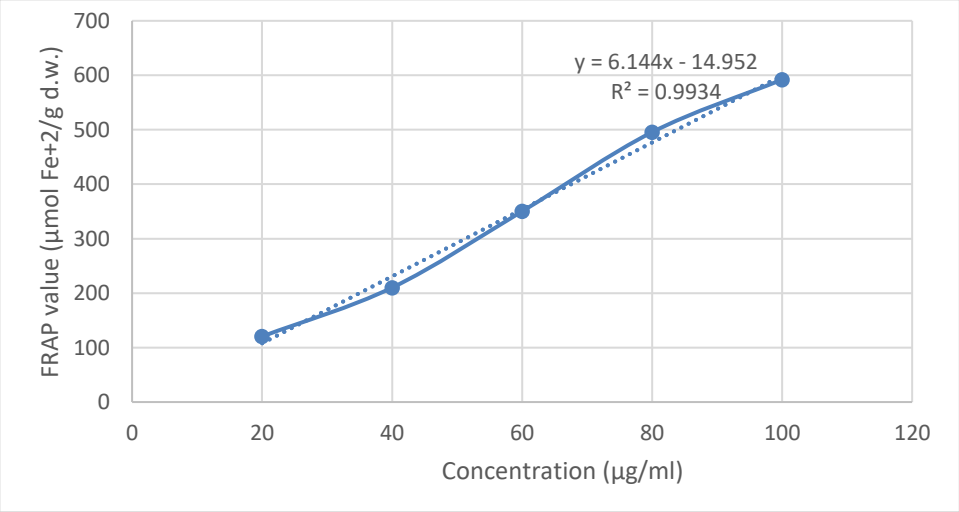
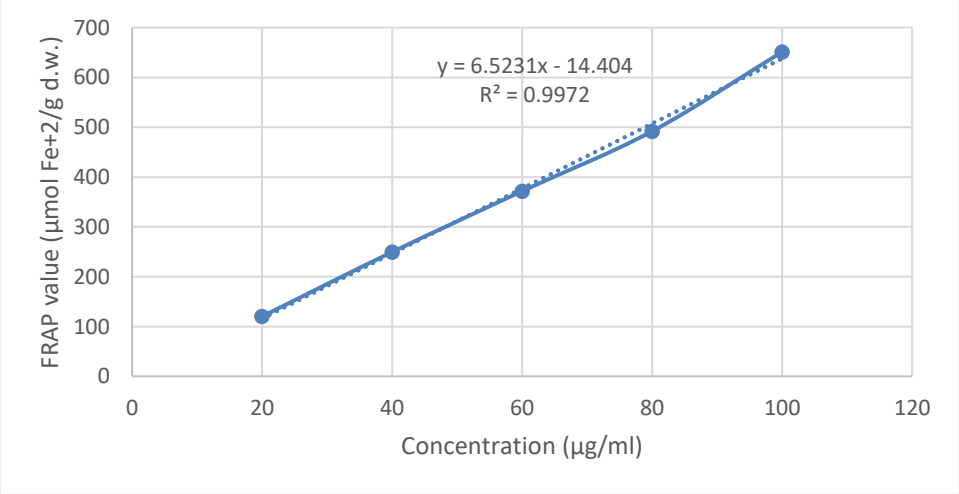


Table 10 FRAP assay activity of Aqueous extract of *Crataeva nurvala*

Concentration (µg/ml)	Absorbance (at 593 nm)	FRAP value (µmol Fe+2/g d.w.)
20	0.098	120.26
40	0.188	249.52
60	0.283	371.78
80	0.376	492.04
100	0.482	651.31
Control	0.059	
IC50 = 9.87		

Graph 3 Graph represents the Percentage Inhibition *Vs* Concentration of Aqueous extract of *Crataeva nurvala*



REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 24, No. 4 (2023)
http://www.veterinaria.org
Article received- 02/10/2023 Revised- 09/11/2023 Accepted- 08/12/2023

3. Nitric Oxide (NO) antioxidant assay

Table 11 Nitric Oxide (NO) antioxidant Assay of Ascorbic acid

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.563	31
40	0.466	43
60	0.333	59
80	0.239	71
100	0.202	75
Control	0.816	
IC50 = 50.00		

Graph 7 Graph represents the Percentage Inhibition *Vs* Concentration of Ascorbic acid

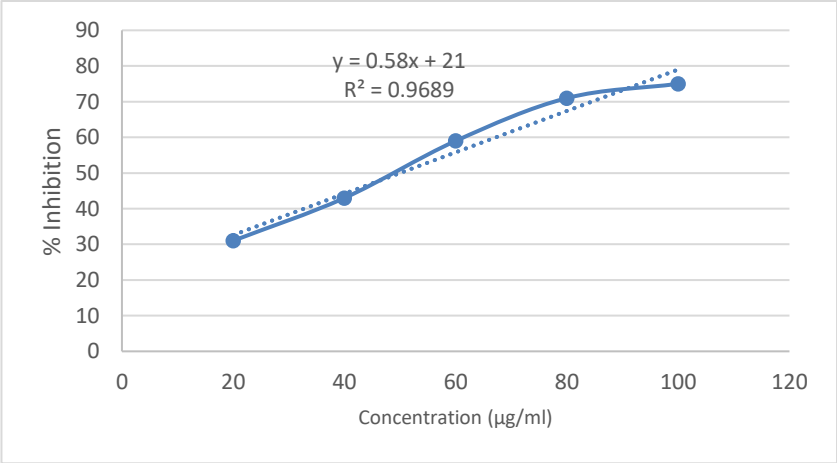
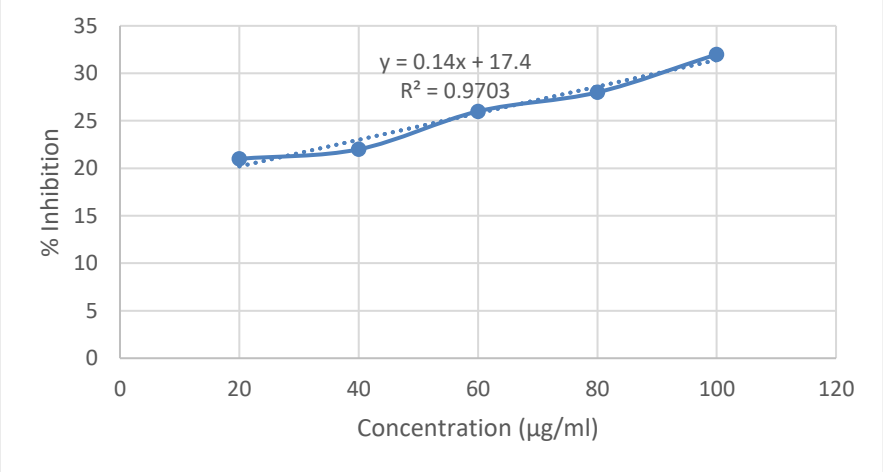


Table 12 Nitric Oxide (NO) antioxidant Assay of Ethanolic extract of *Crataeva nurvala*

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.648	21
40	0.635	22
60	0.603	26
80	0.585	28
100	0.556	32
Control	0.816	
IC50 = 232.86		

Graph 8 Graph represents the Percentage Inhibition *Vs* Concentration of Ethanolic extract of *Crataeva nurvala*

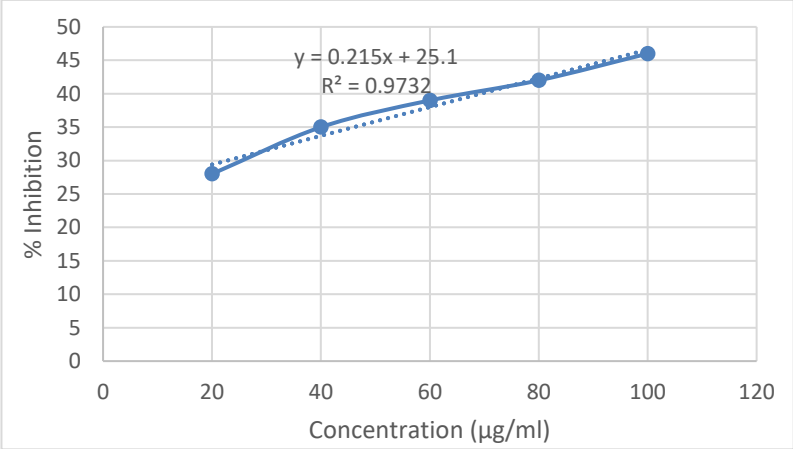


REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 24, No. 4 (2023)
http://www.veterinaria.org
Article received- 02/10/2023 Revised- 09/11/2023 Accepted- 08/12/2023

Table 13 Nitric Oxide (NO) antioxidant Assay of Aqueous extract of *Crataeva nurvala*

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.584	28
40	0.531	35
60	0.495	39
80	0.472	42
100	0.438	46
Control	0.816	
IC50 = 115.81		

Graph 9 Graph represents the Percentage Inhibition *Vs* Concentration of Aqueous extract of *Crataeva nurvala*

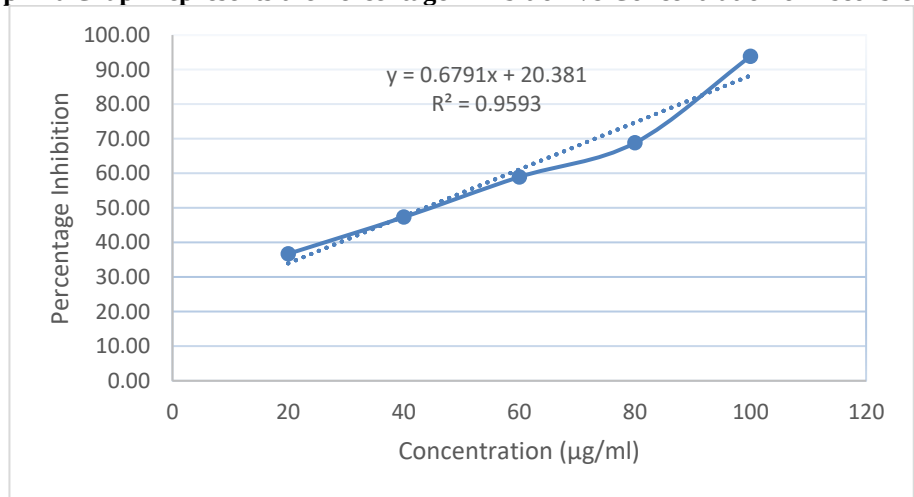


4. Superoxide Radical Scavenging Activity (SRSA) Assay

Table 14 Superoxide radical scavenging activity of Ascorbic acid

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.495	36.70
40	0.412	47.31
60	0.321	58.95
80	0.244	68.80
100	0.048	93.86
Control	0.782	
IC50 = 43.62		

Graph 40 Graph represents the Percentage Inhibition *Vs* Concentration of Ascorbic acid



REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 24, No. 4 (2023)
http://www.veterinaria.org
Article received- 02/10/2023 Revised- 09/11/2023 Accepted- 08/12/2023

Table 15 Superoxide radical scavenging activity of Ethanolic extract of *Crataeva nurvala*

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.618	20.97
40	0.556	28.90
60	0.499	36.19
80	0.454	41.94
100	0.415	46.93
Control	0.782	
IC50 = 106.22		

Graph 11 Graph represents the Percentage Inhibition *Vs* Concentration of Ethanolic extract of *Crataeva nurvala*

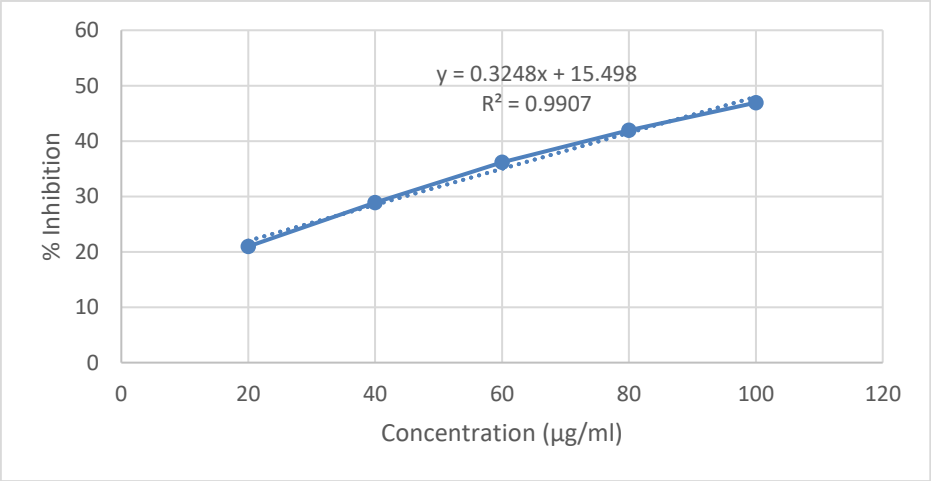
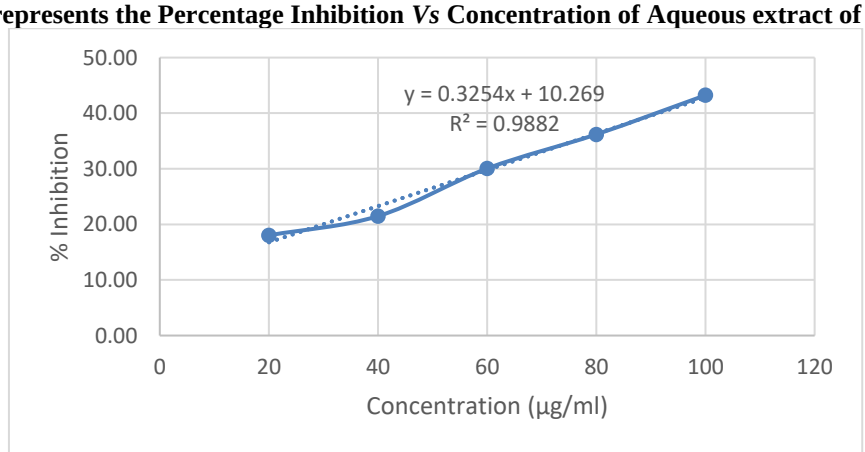


Table 16 Superoxide radical scavenging activity of Aqueous extract of *Crataeva nurvala*

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.641	18.03
40	0.614	21.48
60	0.547	30.05
80	0.499	36.19
100	0.444	43.22
Control	0.782	
IC50 = 122.09		

Graph 12 Graph represents the Percentage Inhibition *Vs* Concentration of Aqueous extract of *Crataeva nurvala*



REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 24, No. 4 (2023)
http://www.veterinaria.org
Article received- 02/10/2023 Revised- 09/11/2023 Accepted- 08/12/2023

Table 17: IC50 values comparison for ECN and ACN Extracts among 4 different Antioxidant activity assay methods:

Extract Name	IC50 value by DPPH Assay	IC50 value by FRAP Assay	IC50 value by NO Assay	IC50 value by SRSA Assay
Ascorbic Acid (Standard)	18.260	5.66	50.00	43.61
ECN	198.604	10.57	232.86	106.22
CAN	169.055	9.87	115.81	122.09
Comparison	Both ECN and ACN have much higher IC50 values than the STD, indicating weaker antioxidant activity. However, ACN (169.055) is slightly better than ECN (198.604).	Both ECN and ACN have higher IC50 values than the STD. However, ACN (9.87) is closer to the STD value, indicating slightly better antioxidant activity than ECN (10.57).	Both samples have higher IC50 values compared to the STD, indicating weaker antioxidant activity. However, ACN (115.81) is better than ECN (232.86).	Both ECN and ACN have higher IC50 values than the STD, indicating weaker antioxidant activity. In this case, ECN (106.22) has slightly better activity compared to ACN (122.09).

Summary and Conclusion:

The comprehensive study of *Crataeva nurvala* leaves—covering morphology, microscopic analysis, physico-chemical properties, microbial quality control, and fluorescence behavior—provides valuable insights. Morphological analysis reveals the plant's external structure and adaptations, while microscopic examination of transverse sections uncovers internal anatomy and vascular arrangements, aiding in species identification and understanding plant functions. Physico-chemical and microbial analyses ensure the leaves' safety and efficacy for medicinal use. Furthermore, fluorescence analysis of powdered leaves with various reagents helps authenticate the material and detect adulteration, enhancing the quality control of herbal products. Phytochemical screening of *Crataeva nurvala* leaves revealed that it contains carbohydrates, alkaloids, glycosides, phenolic compounds, flavonoids, steroids, saponin and tannins, with varying presence across different extracts. As Phenolic compound, Flavonoids and Tannins were present in ethanol and water (polar solvents) and these secondary metabolites were absent in petroleum ether and ethyl acetate (non - polar solvents). Hence, ethanolic and aqueous extract of *Crataeva nurvala* were selected for further antioxidant activity study. The distribution of these compounds can provide insight into the medicinal and pharmacological potential of *Crataeva nurvala*. Based on the result of the antioxidant activity study by four different assay methods, it can be concluded that the leaves extract

- ACN has a better IC50 value than ECN in the DPPH, FRAP, and NO assays, indicating stronger antioxidant activity in these tests.
- ECN only outperforms ACN in the SRSA assay, but the difference is not substantial.

ACN shows overall stronger antioxidant activity compared to ECN when considering all four assays. Therefore, ACN is the sample that shows the best antioxidant result compared to the standard (STD) across these assays.

Its potential for medicinal applications is further supported by its antioxidant activities, making it a subject of interest in ongoing research. The plant's adaptability to various environments and its therapeutic versatility contribute to its significance in both traditional and modern medicine.

Abbreviations

CN - *Crataeva nurvala*, ECN – Ethanolic extract of *Crataeva nurvala*, ACN – Aqueous extract of *Crataeva nurvala*, SNP - Sodium nitroprusside, STD – Standard, DPPH - 2,2-Diphenyl-1-picrylhydrazyl, FRAP - Ferric Reducing Antioxidant Power, NO - Nitric Oxide, SRSA - Superoxide Radical Scavenging Activity.

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