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Unveiling the Synoptic Research of in Vitro Pharmacological Activities in Methanolic Extracts of Both Fruits and Roots of Coccinia Grandis

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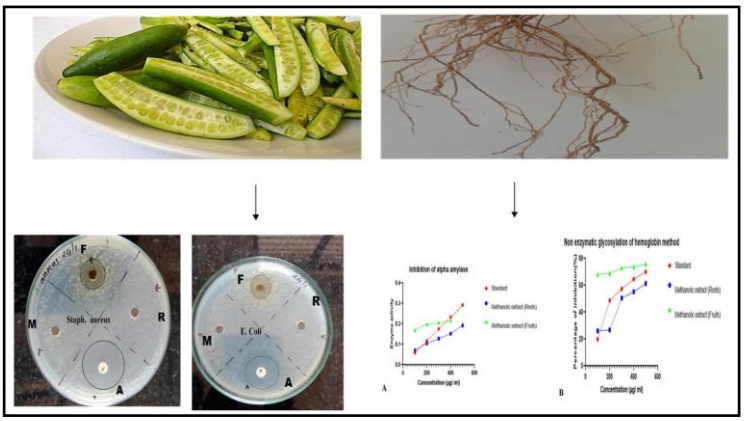
KEYWORDS

Coccina grandis, anti-inflammatory, anti-microbial, anti-oxidant, cytoprotective, anti-diabetic.

ABSTRACT:

Ethnopharmacological relevance: Coccinia grandis is well known for its curative properties and used as folk medicine in India and other countries due to its various pharmacological characteristics due to presence of number of phytochemicals.
Aim: The aim of the present study was to evaluate pharmacological properties of the fruits and roots of Coccinia grandis.
Materials and methods: The methanolic extracts of these parts were tested and analyzed for antibacterial, anti-inflammatory, anti-oxidant, cytoprotective and antidiabetic properties. The antibacterial activity of both extracts was tested against two strains of E.coli and Staphylococcus aureus using agar diffusion method. The in vitro anti-inflammatory activity was done using RBC membrane stabilization method and inhibition of protein denaturation method. The anti-oxidant analysis was done using DPPH, FRAP, SOD, nitric oxide radical scavenging assay. Cytoprotective activities were carried out by MTT assay in 24 hr – HeLA cells. The anti-diabetic activity was done by inhibition of alpha amylase method and non enzymatic glycosylation of hemoglobin method. **Results:** The methanolic extracts of both roots and fruits had showed variety of phytochemicals alongwith antioxidant, antibacterial, anti-inflammatory, anti-diabetic and cytoprotective activities. However, fruit extract showed greater activities as compared to root extract.
Conclusion: Methanolic fruit extract can serve as promising source for various pharmacological activities.

Graphical abstract



Highlights:

- **Coccinia grandis is well known for its curative properties and used as folk medicine in India and other countries**
- **Estimation of high phenol and flavonoids from methanolic extracts of fruits and roots**
- **Excellent antioxidant, anti-inflammatory, anti-diabetic, cytoprotective potential in methanolic extract of fruits**
- **Fruit extract can be serve as promising pharmacological agent for future research studies**

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1. Introduction:

Hippocrates mentioned "Nature itself is the best physician". Traditional system of medicines propound that plants have a significant role in maintaining human health and improving the quality of human life **(Ross, 2007)**. The Cucurbitaceae family is commonly known as gourd, melon and pumpkin family **(Killon, 2000).** Coccinia grandis (Ivy gourd) also called baby water-melon, belonging to Cucurbitaceae family, is used as vegetable and grown throughout Indian subcontinent. They are deciduous and usually grown in wooden grasslands, semi-arid forests, coastal areas and highlands **(Tamilselvan et al., 2011)**. The plant part of C. grandis such as roots leaves and fruits are used for numerous medicinal purposes like wound healing, ulcers, jaundice, diabetes **(Rahmatullah et al., 2009)** and antipyretic analgesic, anti-inflammatory, anti-tussive, antioxidant, anti-mutagenic, antibacterial, anti-protozoal, hepatoprotective, expectorants **(Potterat, 1997).** The presence of phenols, tannins, saponins, terpenoids , flavonoids, arabinose, xylose, mannose, galactose, glucose and rhamnose can be detected in phytochemical samples. Plant extract tests, particularly leaf extract, show that it has antihyperglycemic, inhibitory xanthine oxidase, analgesic, anti-inflammatory, antipyretic, antioxidant, antihyperlipidemic, antimicrobial , anti-hepatotoxic and anti-insecticide activities **(Ramachandran et al., 2015)**. Antibacterial, antioxidant and cell proliferative properties of Coccinia grandis fruits were studied and concluded that there were certain degrees of antimicrobial, antioxidant and cell proliferative properties in the grandis fruits. Their study was mainly focused on the extract obtained from fruits **(Sakharkar et al., 2017)**. Similarly, phytochemical and pharmacological screening of Coccinia grandis Linn was done and concluded that the ethanol extract possesses the analgesic as well as antirheumatic properties **(Hossain et al., 2014)**. Considering all these potential pharmacological properties and presence of various phytochemicals like tannins, saponins, flavonoids etc we conducted the screening to investigate the phytochemical constituents responsible for anti-inflammatory, antibacterial, anti-oxidant, anti-diabetic and cytoprotective activities of the fruits and roots extract. The comparative analysis was also done to ensure more understanding for the future pharmacological research. Also in recent years, investigation of various plant based lead molecules, screening of metabolites from traditional plant based medicine throw light on pharmaceutical sector in the developing novel drugs **(Meckes et al., 1996).** Thus this study identifies different pharmacological properties which could be used to develop infusions, nutriceuticals and pharmaceuticals.

2. Materials and methods

2.1 Materials:

The organic fruits and roots of Coccinia grandis were collected from Kerala Agricultural University Vellayani (Trivandrum).

2.2 Methods:

2.2.1 Sample preparation (Drying and grinding):

The fruit and root samples were collected were washed properly under running water to remove soil and other debries. The washed samples were blotted using dry tissue paper to remove adhered water and cut in to small slices and pieces of fruit and roots respectively. These samples were dried at 40 ^{0}C in solar dryer . The dried samples were then ground to fine powder using mixer grinder and stored in air tight container at refrigerator temperature till further use.

2.3 Methanol Extraction

The dried samples, preserved in refrigerator were kept in hot air oven for 10 minutes to remove the adhered moisture and immedietky used for extraction. Nearly 25 g of the dried powder were extracted with methanol for overnight using Soxhlet apparatus. The methanol extract was used for further phytochemical studies and in vitro studies **(Singh et al., 2018)**

2.4 Phytochemical analysis:

The methanolic extract was used for preliminary phytochemical screening of alkaloids (Meyer's tests), carbohydrates (Molish's,Benedict's and Fehling's test) **(Osborne et al., 1903)**,
saponins (foam and froth tests) tannins (gelatin test), flavonoids (Alkaline reagent and lead acetate tests **(Ganesh et al., 2014).** The screening was done as per the standard methods including tests for alkaloids, gums, reducing sugar, tannins, flavonoids, saponins; terpenoids **(Meckes et al., 1996)** were analyzed.

2.5 Estimation of total phenolic content:

The total phenolic content was estimated quantitatively based on conventional Folin-Ciocalteu reagent assay **(Patel et al., 2012).** The standard solution of gallic acid (100 mg/ml) along with the 1ml extract and 9 ml distilled water was added to volumetric flask.1ml of Folin-Ciocalteu reagent and 10 ml of 7% Na2CO3 solution were added with 5 min difference and and volume was made to 25 ml using distilled water and mixed properly. After incubation for 90 min at room temperature, the absorbance was measured against prepared reagent blank (distilled water) at 750 nm in UV-vis spectrophotometer. Total phenolic content of root extracts expressed as mg gallic acid equivalents

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(GAE)/100 G fresh weight **(Bhandary et al., 2012)**. The phenolic content was determined in triplicate, results were taken in average and data was reported as mean ± SD.

2.6 Estimation of total flavonoids content:

Total flavonoids content was determined quantitatively using aluminum chloride colorimetric assay **(Vábková et al., 2013).** The standard solution of catechin (100 mg/ml), 1ml methanolic extract and 4ml distilled water were added to 10 ml volumetric flask. Then 0.3 ml 5 % $NaNO_2$ and 10 % $AlCl_3$ were added with a time difference of 5 min. After 1 min, 2ml of 1M NaOH was added and the total volume was diluted to 10 ml with distilled water. The solution was mixed well and the absorbance against prepared reagent blank (distilled water) was determined at 510 nm with an UV-Vis Spectrophotometer. The total flavonoids content of root extracts expressed as mg catechin equivalents (CE)/100 G fresh weights. The flavonoids content was determined in triplicate, results were taken in average and data was reported as mean ± SD.

2.7 Antioxidant potential test:

2.7.1 (**2, 2-diphenyl-1-picryl-hydrazyl-hydrate) DPPH method:**

The plant extracts of concentrations ranging from 250-1500 µg/ ml was taken in test tubes. 0.5 ml of DPPH from the prepared stock of 200 mM DPPH in 95% methanol was kept 30 mins at room temperature **(Sun et al., 2012).** The optical density was measured at 517nm and stranded ascorbic acid was used as reference.

Calculation: - % of inhibiting is calculated by using formulae

Percentage inhibition = (Ac- As/Ac) × 100

Ac: control of absorbance

As: Sample absorbance

2.7.2. Ferric ion Reducing Antioxidant Power assay (FRAP)

The ferric reducing assay power was determined by the Fe3+ to Fe2+ transition and Fe2+ is observed by measuring the Pesl's Prussian blue formation at 700 nm. The varying concentration (1-5 mg/ml) of plant extract, 2.5 ml phosphate buffer (pH 6.6), 2.5ml of 1% potassium ferricyanide were mixed together and incubated at 50°c for 30 mins **(Okoko et al., 2012).** Then 2.5 ml of 10% Trichloroacetic acid (TCA) is added, mixed and centrifuged at 3000 rpm for 10 min. The supernatant (2.5 ml), 2.5 ml H2O, 0.5 ml 1% ferric chloride are mixed and subjected to absorbance at 700nm in UV- visible spectrometer. All the tests were done in triplicate, Standard Butylated hydroxy toluene (BHT) used as reference and expressed as IC50 **(Katalinic et al., 2006)**

Calculation :-

Percentage increase in reducing power = [As/Ab] × 100

As: Sample absorbance

Ab: blank absorbance

2.7.3 Nitric oxide radical scavenging assay:

Concentration ranging from 250-1500 µg/ ml dissolved in 5mM sodium nitroprusside and phosphate buffers (0.025M pH 7.4) were incubated at 25°c for 150 min. Then, 0.5 ml aliquot of incubated sample was removed and mixed with 0.5 Griess reagent. The absorbance of chromophore formed was read at 546nm, repeated in triplicates and standard curcumin used as reference **(Abas et al., 2006)** and scavenging activity was calculated using following formula

N2O scavenging activity (€o) = [(Ac-At)/Ac] × 100

Ac- Absorbance of control

At- Absorbance of test sample

2.7.4 Super oxide dismutase (SOD) anion radical scavenging assay:

In this method the reaction mixture is prepared with phosphate buffer (pH 7.8), NBT, EDTA, Riboflavin etc. Make up to 3 ml including the varying concentration of extract from 250-1500µg/ ml. The tubes were incubated for 15 min and based on generation of super oxide radical in presence of light by auto oxidation of riboflavin. Then colored formazon is measured at 560nm and IC50 values calculated with standard ascorbic acid **(Mohan et al., 2012)** and scavenging activity was calculated using following formula

Percentage Reduction = [(Ac-At)/Ac] × 100

Ac- Absorbance of control

At- Absorbance of test sample

2.7.5 Hydrogen peroxide scavenging assay:

The varying concentration from 250-1500 µg/ ml was taken in different test tubes. 1ml of H2O2 was added to all the test tubes followed by incubation at room temperature for 10 min. H2O2 is prepared with the standard buffer (pH 7.4). The absorbance value of the reaction mixture was noted at 700 nm. IC50 values are compared with standard, α-tocopherol **(Özyürek et al., 2010)** and scavenging activity was calculated using following formula

[(Ac-At)/Ac] × 100

Ac- Absorbance of control

At- Absorbance of test sample

2.8 Anti bacterial test:

The antibacterial potential of extracts was determined using agar diffusion method against gram positive and negative bacteria **(Somchit et al., 2003)**. The plates were spread with 0.6 ml of subcultured bacterias i.e Staphylococus aureus and E.coli. The wells were made using cork bore and 10µL of sample and streptomycin

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(antibiotic) were added and kept for incubation for 24 hours at….. for 24 hours **(Al-Bayati et al., 2008).** The zone of inhibition were recorded for both extracts.

2.9 Anti-inflammatory activity:

2.9.1 RBC membrane stabilization method (Anosike et al., 2012)

For this study, the blood (10 ml) was collected from healthy volunteers. Blood was transferred aseptically to the heparinized centrifuged tube. The collected blood was mixed with equal volume of Alsever solution (dextrose 2%, sodium citrate 0.8%, citric acid 0.05%, sodium chloride 0.42%, and distilled water 100 mL) . The tubes were centrifuged at 3000 rpm for 10min and were washed three times with equal volume of isosaline. Various concentrations of extracts were prepared (100μg/mL – 500 μg/mL) incubated at 37ºC for 30 min and centrifuged at 3000 rpm for 20 min. The absorbance of the supernatant solution was read at 560nm. The percentage hemolysis was calculated using the following formula.

Percent of hemolysis = (At /Ac) × 100

Anti inflammatory Activity (%) = 100 – ((At /Ac) × 100).

At - test sample's absorbance

Ac - absorbance of the negative control (Alsever's solution with blood in it & without Aspirin or Methanolic plant extract in it).

2.9.2 Inhibition of Protein Denaturation

The inhibition of protein denaturation test was performed to determine the anti-inflammatory potential of methanolic extracts of the Coccinia grandis. For this, test extracts of varying concentration and 1% aqueous solution of BSA (Bovine serum albumin) are mixed well and incubated at 37˚C for 20 min. Then it was heated to 51˚C for 20 min. Measure the absorbance at 660nm using UV-Visible Spectrophotometer and performed in triplicates **(Wallner et al., 1986)**. The percentage inhibitions was calculated by using following formula

% Inhibition = ((Ac-As)/Ac)×100

At - test sample's absorbance

Ac - absorbance of the negative control (Alsever's solution with blood in it & without Aspirin or methanolic plant extract in it).

2.10 Anti diabetic potential testing:

2.10.1 Standard maltose curve:

The antidiabetic potential of methanolic extracts were evaluated by standard maltose curve.

Nearly 0.2-1 ml of standard maltose (1mg/ml) solution was taken into different test tubes. Distilled water was added to each test tube to make the volume to 1.0 ml. DNS reagent of 0.1 was added to each test tube and all the test tubes were then placed in boiling water bath for 15 minutes. 8.0 ml of distilled water was added to each test tube and the contents of the tubes were mixed thoroughly. Then, the absorbance of the solution was determined using calorimeter at 570 nm against blank solution **(Ali et al., 2006).**

2.10.2. Alpha amylase inhibition Assay: (Oyedemi et al., 2017)

Methanolic extract of 100-500 µl (100-500 µg) was added in different test tubes. Phosphate buffer of 6.9 pH was added to test tubes containing methanolic extracts to make the volume to 0.5 ml. The measurements of the blank were done with 1 ml of phosphate buffer and control with 0.5 ml of phosphate buffer. The obtained solutions were treated with 0.5 ml of alpha amylase at concentration of 0.5 mg/ml. The resulting solutions were incubated at 25°C for the period of 10 minutes. Then 0.5 ml of 1% starch solution was added in 0.02 M sodium phosphate buffer of pH 6.9 to all the tubes, and then incubated at 25°C for 10 minutes. The reaction was stop by adding 1.0 ml of DNS and the reaction mixture was kept on boiling water bath for 5 minutes and then it was allowed to cool to room temperature. The solution was mixed with 8 ml of distilled water. The absorbances measured by calorimeter at 570 nm against blank solution. The amount of maltose produced was calculated using using standard maltose curve. The enzyme activity was calculated by using following formula:

Enzyme Activity = Amount of Maltose formed x 210 x 342] [Kim GN, etal, 2009]

2.10.3 Non-enzymatic glycosylation of hemoglobin assay (Ghosh et al., 2012)

Glucose (2%), Haemoglobin (0.06%) and Gentamicin (0.02%) solutions were prepared in phosphate buffer 0.01 M, pH 7.4. Mixture of 1 ml each of the above solutions was made and 1 ml of each concentration was added to above mixture. The solution was incubated at room temperature in dark condition for 72 hours. The degree of glycosylation of haemoglobin was measured in calorimeter at 520 nm. Alpha - Tocopherol (Trolax) was used as a standard drug for assay.

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3. Results and discussion

3.1 Phytochemical analysis and quantitative estimation of phenol and flavonoids contents:

Coccinia grandis fruits and roots were collected, dried properly and methanolic extracts were prepared as discussed in methodology section and those were subsequently screened for phytochemical analysis. Phytochemical analysis of extract revealed the presence of carbohydrates, saponins, alkaloids, flavonoids, tannins, terpenoids, gums, phenols **(See table 1).** These observations clearly indicated the potential use of fruits and roots in various disorders due to presence of number of phytochemicals. The fruits and roots didn't show the presence of oil which was confirmed from paper press test. It has been observed that total phenolic content was found to be maximum in fruits (13.3±0.01 mg/g.d.wt.) followed by root (9.833±0.01mg/g.d.wt.). Similar results were observed in case of total flavonoids contents i.e. total flavonoids content was found to be maximum in fruits (72.5±002 mg/g.d.wt.) followed by roots (62.33±0.001 mg/g.d.wt.).The methanolic extract was subjected to various in vitro analysis including anti-inflammatory, anti-bacterial, anti-oxidant, anti-diabetic and cytoprotective activities were also tested for both roots and fruits separately. Thus comparative analysis is also done.

Table 1: Phytochemical screening of methanolic extracts of Coccinia grandis

Sample	Observation	Inference	Fruits	Roots
Benedict test	Brick colored precipitate	Presence of carbohydrates	+	+
Fehling's test	Brick colored precipitate	Presence of carbohydrates	+	+
Molish's test	Formation of violet ring	Presence of carbohydrates	+	+
Test for saponin using olive oil	Foam appearance	Presence of saponins	+	+
Mayers's test	Yellow precipitate	Presence of alkaloid	+	+
Dragendorff's test	Reddish brown Precipitate	Presence of alkaloid	+	+
Hagers test	Yellow colour formation	Presence of alkaloid	+	+
Wagners test	Yellow precipitate	Presence of alkaloid	+	+
Alkaline reagent test	Yellow colour formation	Presence of flavonoids	+	+
Lead acetate test	Yellow colour formation	Presence of flavonoids	+	+
Shinoda test	Magenta colour formation	Presence of flavonoids	+	+
Zinc HCl test	Magenta colour formation	Presence of flavonoids	+	+
Ferric chloride test	Bluish black precipitate	Presence of phenol	+	+
Gelatin test	Formation of white precipitate	Presence of tannins	+	+
Filter paper press test	No formation of oily stain	Absence of fat and fixed oil test	-	-
Salkowski's test	Golden yellow colour formation	Presence of triterpenes(phytosterol)	+	+
Ninhydrin test	Blue colour formation	Presence of amino acids	+	+

+ indicates the presence of that component, - indicates the absence of that component

3.2 Antioxidant potential of methanolic extract s of roots and fruits:

The antioxidant potential of methanolic extracts of Coccinia grandis fruits and roots were evaluated for antioxidant potential using various methods described in methodology section. DPPH scavenging assay is an important assay to determine the antioxidant activity of the plant extracts in in vitro model **(Mensor et al., 2012).** DPPH is free radical which reacts rapidly with the antioxidant compounds **(Scherer et al., 2009)**. The antioxidant compounds can donate a hydrogen atom to DPPH and change the color. The intensity of color was measured calorimetrically. The increasing intensity of color is directly proportional to the inhibition of DPPH. The present study showed the concentration dependent activity for DPPH inhibition. The maximum inhibition was noticed at 75.133% for fruit and 42.62% for root (see table 2).

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Table 2: DPPH scavenging potential of methanol extracts of Coccinia grandis fruits and roots

Sr. No	DPPH Concentration (µg/ ml)	250	500	750	1000	1500	IC50
1	% inhibition fruits	56.3	64.2	76.4	85.3	90	225
2	% inhibition roots	55.55	62.5	75.3	82.7	89.5	229
3	% inhibition standard	57.5	68.9	79.5	88.2	91.5	220

Figure 1 describes the comparative antioxidant potential of methanol extracts of Coccinia grandis fruits and roots and standard. It was clearly concluded that the fruits had excellent and comparable antioxidant activity in comparison to standard.

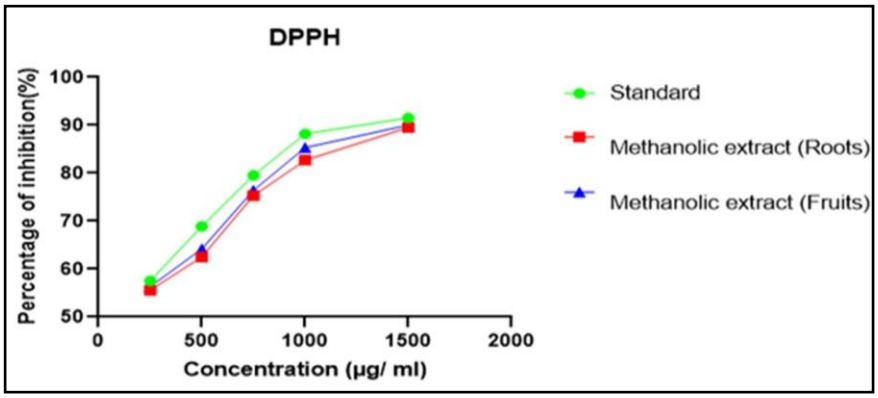

Fig.1: Comparative DPPH scavenging activity of methanol extracts of Coccinia grandis fruits and roots

Similarly the antioxidant potential was determined by FRAP assay. The reducing power is related to electron transfer ability of the plant extract. In this assay the transfer capacity of Fe3+ to Fe2+ was measured **(Guo et al., 2003)**. The results showed that the extract possessed antioxidant activity in a concentration dependent manner. Based on the results the C.grandis have a ability of transferring the Fe3+ in to Fe2+ , and it minimize the oxidative damage in the tissues **(See table 3)**.

Table 3: FRAP assay of methanolic extracts of Coccinia grandis fruits and roots

Sr. No	FRAP Concentration (µg/ ml)	250	500	750	1000	1500	IC50
1	% inhibition fruits	28.5	40	51.2	60.7	70.3	750
2	% inhibition roots	26.66	31.12	40	57.77	62.23	780
3	% inhibition standard	30	47.7	58.12	63.17	75.3	720

Figure 2 describes the comparative antioxidant potential of methanol extracts of Coccinia grandis fruits and roots and standard. It was clearly concluded that the fruits had excellent and comparable antioxidant activity in comparison to standard.

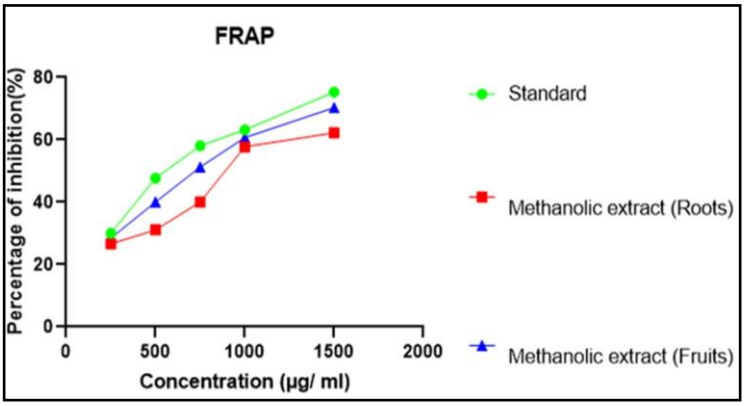

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Fig.2: Comparative antioxidant activity of methanol extracts of Coccinia grandis fruits and roots using FRAP assay method

Nitric oxide radical can be determined by the principle of Griess illosvoy reaction **(Abas et al., 2006)**. Here, in aqueous solution at physiological pH sodium nitroprusside help in creating nitric oxide inclines with oxygen to form nitrite ions. This is measured by griess reaction. Nitric Oxide is a free radical which is formed from sodium nitroprusside and it reacts with oxygen to form nitrite. The antioxidant activity was measured by the inhibition of the nitrite formation, this was done by the plant extracts which directly reacts with oxygen, nitric oxide and other nitrogen compounds. The present study proves that the increasing concentration of the extract have a maximum inhibitory activity against the nitric oxide **(See table 4)**.

Table 4: No assay of methanolic extracts of Coccinia grandis fruits and roots

Sr. No	NO assay (µg/ ml)	250	500	750	1000	1500	IC50
1	% inhibition fruits	17.5	26.23	39.8	59	71.7	838
2	% inhibition roots	16.2	25.1	38.9	58	70.4	852
3	% inhibition standard	18	27	41	60	72	820

Figure 3 describes the comparative antioxidant potential of methanol extracts of Coccinia grandis fruits and roots and standard. It was clearly concluded that the fruits had excellent and comparable antioxidant activity in comparison to standard.

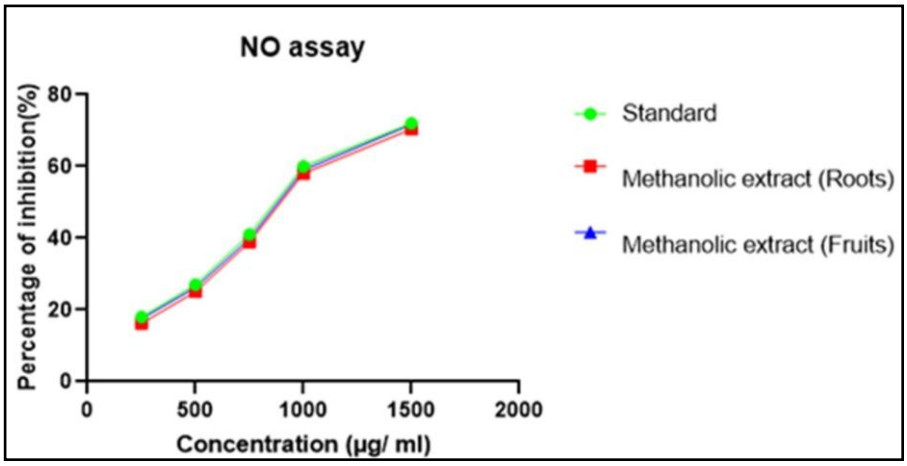

Fig.3: Comparative antioxidant activity of methanol extracts of Coccinia grandis fruits and roots using NO assay method

Superoxide dismutase is an important enzyme in an antioxidant defense system **(Kaushik et al., 2003)**. SOD converts the superoxide anion into hydrogen peroxide and thus reduces the toxic effect. The percentage of inhibition of superoxide by SOD may reduce the cellular damages. The present study proves that the increasing concentration of the extract have a maximum inhibitory activity of SOD.

Table 5: No assay of methanolic extracts of Coccinia grandis fruits and roots

Sr. No	SOD assay (µg/ ml)	250	500	750	1000	1500	IC50
1	% inhibition fruits	37	48.2	65	81.2	90.5	590
2	% inhibition roots	36	46.8	63.9	80.5	89.7	600
3	% inhibition standard	38.5	49.1	66	82	91	580

Figure 4 describes the comparative antioxidant potential of methanol extracts of Coccinia grandis fruits and roots and standard. It was clearly concluded that the fruits had excellent and comparable antioxidant activity in comparison to standard.

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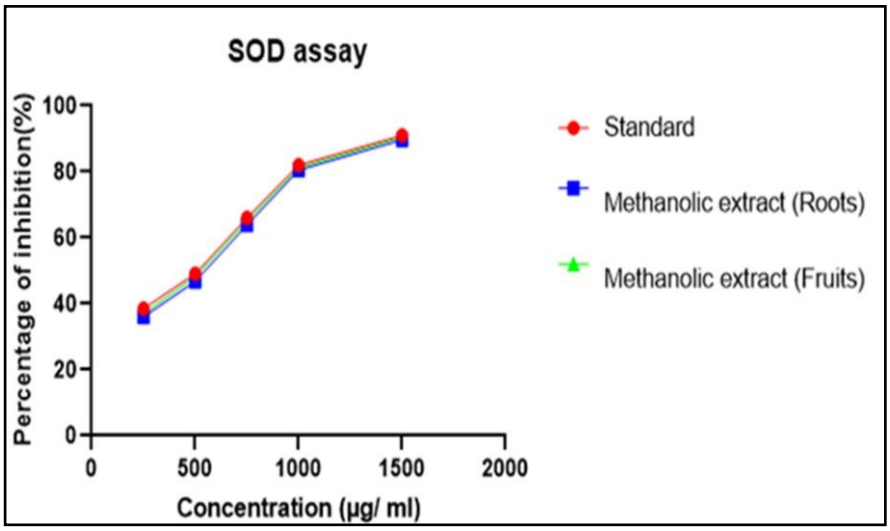

Fig.4: Comparative antioxidant activity of methanol extracts of Coccinia grandis fruits and roots using SOD assay method

Hydrogen peroxide (H2O2) is a non-radical reactive oxygen species (ROS) that is biologically essential and can affect many cellular processes **(Gechev et al., 2006)**. We found that the in vitro H2O2 scavenging behavior of methanolic extracts can be observed in our assay.

Table 6: Assay of methanolic extracts of Coccinia grandis fruits and roots

SR. No	H2O2 scavenging assay (µg/ ml)	250	500	750	1000	1500	IC50
1	% inhibition fruits	68.5	71	73.1	80.2	86.3	200
2	% inhibition roots	67.2	70	71.5	80.5	85.2	205
3	% inhibition standard	70	72.2	73.5	81.5	87.5	180

Figure 5 describes the comparative antioxidant potential of methanol extracts of Coccinia grandis fruits and roots and standard. It was clearly concluded that the fruits had excellent and comparable antioxidant activity in comparison to standard.

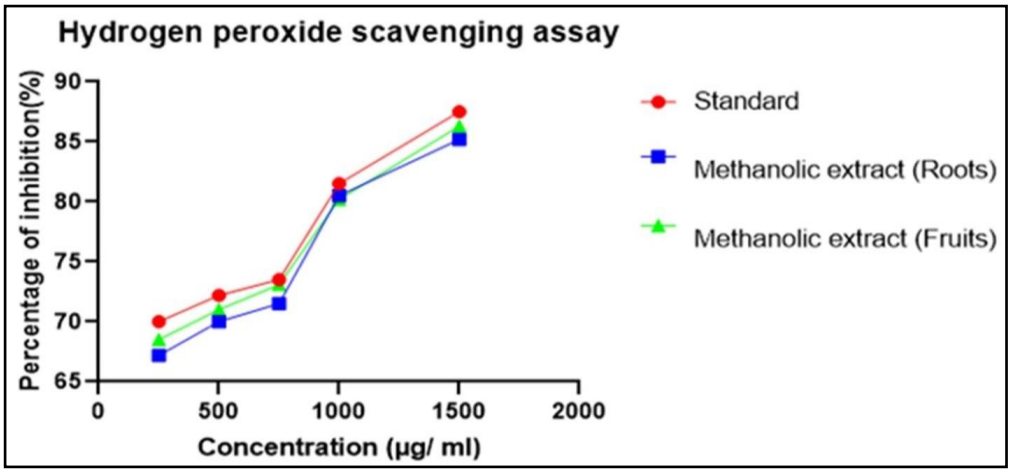

Fig.5: Comparative antioxidant activity of methanol extracts of Coccinia grandis fruits and roots using H2O2 assay method

3.2 Antibacterial potential of methanolic extract s of roots and fruits:

The antibacterial potency of methanolic extract of roots and fruits were determined against both gram positive and gram negative bacterias. In this study, the C. grandis

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exerted antibacterial activity against both strains of the micro organisms. The C. grandis plant was used for testing their antibacterial activity and showed high activity against those organisms and these indicate that the herbal preparations could be used for preventing and treat the diseases caused by those selected organisms. The results revealed that the concentration of extract showed antimicrobial activity. However maximum zone of inhibition was observed in fruits while using Staphylococcus aureus gram positive bacteria **(see table 7 and figure 6)**. However, further investigations of its activity against a wider range of bacteria and identification purification of its chemical constituents, and toxicological investigations of the plant extracts should be carried out with a view to developing novel drugs for human consumption.

Table 7: Comparative antimicrobial activity of methanolic extracts of Coccinia grandis fruits and roots

Test	Organisms	Root zone of inhibition(mm)	Fruit zone of inhibition(mm)
Antibacterial	Staphylococcus aureus	No zone	7.0
Antibacterial	E.coli	No zone	5.5

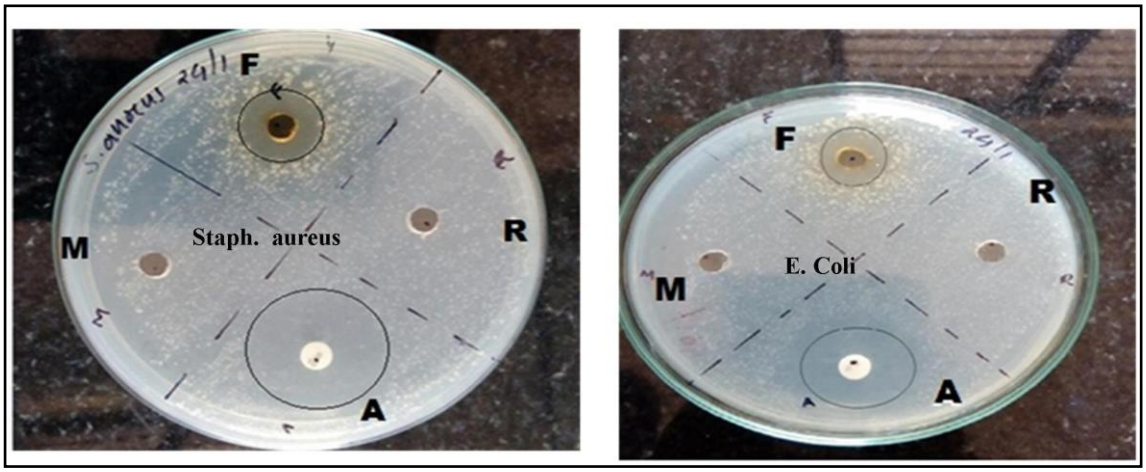

Fig.6: Comparative zone of inhibition (ZOI) of methanol extracts of Coccinia grandis fruits (F),roots (R), antibiotics Streptomycin (A) and control sample methanol (M) against gram positive and negative bacterias

It can be observed from the figure 5 that the fruit extract had sown excellent ZOI against both gram positive and negative microorganisms while root extract didn't showed any sign of inhibition against both types of bacterias.

3.4 Anti-inflammatory potential of methanolic extract s of roots and fruits:

The anti-inflammatory activity of extracts was determined using RBC stabilisation method and protein denaturation method. The result revealed that all the varying concentrations of extracts showed the wide range of anti-inflammatory activity. However, the maximum activity (%) is shown by the fruits than roots when compared with the aspirin but still both fruits and roots could be a promising anti inflammatory agent. In both methods fruit extract showed greater anti-inflammatory activity as compared to root extracts.In RBC membrane stabilization method, fruit extract showed 50-85 percentage of anti-inflammatory activity while root extract showed only 14-45 percentages. The similar trend was observed in protein denaturation method **(See table 8 and Fig. 7)**. These results clearly indicated the potential of fruit extracts as promising anti-inflammatory agent. The probable reason for maximum anti-inflammatory activity of fruits extracts might be due to higher phenolic and flavonoids contents obtained in our research work.

Table 8: Comparative antimicrobial activity of methanolic extracts of Coccinia grandis fruits and roots by RBC membrane stabilization and protein denaturation method

Method	RBC membrane stabilisation		Protein denaturation	
Concentration(µg/ml)	% AI (Fruit)	% AI (Root)	% AI (Fruit)	% AI (Root)
100	50	13.34	65	15
200	62.5	20	78.3	20
300	72.5	33	81.6	31.6
400	80	37.77	83	41.6
500	85	46.67	85	48.3

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Aspirin	87.5	67.78	87	82.2
Negative control	0	0	0	0

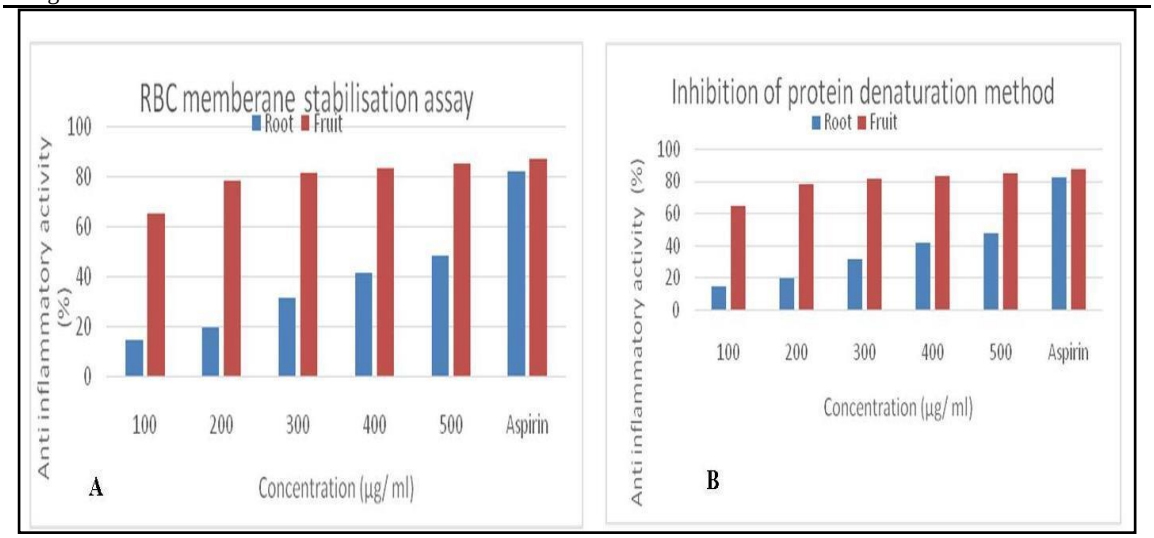

Fig.7: Comparative anti-inflammatory activity of methanolic extracts of fruits and rots using RBC membrane stabilizing assay (A) and Protein denaturation inhibition (B). In both methods fruit extract showed higher anti-inflammatory activity proving promising agent

3.5 Anti-diabetic potential of methanolic extract s of roots and fruits:

The anti-diabetic potential of methanolic extracts of C. grandis fruits and roots were determined by using alpha amylase inhibitory assay and non enzymatic glycolisation of hemoglobin assay. The methanolic extract of C. grandis fruits had in vitro antidiabetic activity in a dose dependent manner by the inhibitory activity of alpha amylase and non enzymatic glycolisation of hemoglobin. The present research has been carried in the fruit and roots of Cocciniagrandis and their effect in inhibiting glycosylation of haemoglobin and alpha-amylase. The inhibition of the activity of alpha-amylase and alpha-glucosidase would delay the degradation of carbohydrate, which would in turn cause a decrease in the absorption of glucose, as result the reduction of postprandial blood glucose level elevation. The present study reveals that C.grandis possess anti-diabetic manner in a concentration dependent manner for fruits but for roots it's comparatively lower (See table 9 and 10)

Table 9: Alpha amylase activity of methanolic extracts of roots, fruits and standard

Standard		Methanolic extract (root)		Methanolic extract (Fruit)	
Con	Enzyme activity	Con	Enzyme activity	Con	Enzyme activity
100	0.058	120	0.07	290	0.169
200	0.116	180	0.105	340	0.198
300	0.175	220	0.128	350	0.2046
400	0.233	260	0.152	370	0.216
500	0.292	330	0.192	440	0.257

Table 10: Non-enzymatic glycolisation of hemoglobin assay

Concentration(µg/ml)	Standard	Methanolic extract (Root)	Methanolic extract (Fruit)
		% Inhibition	
100	19.8	26.1	68
200	48.6	26.7	68.9
300	57.2	50.4	72.8
400	64.5	55.1	73.7
500	70.0	61.3	75.7

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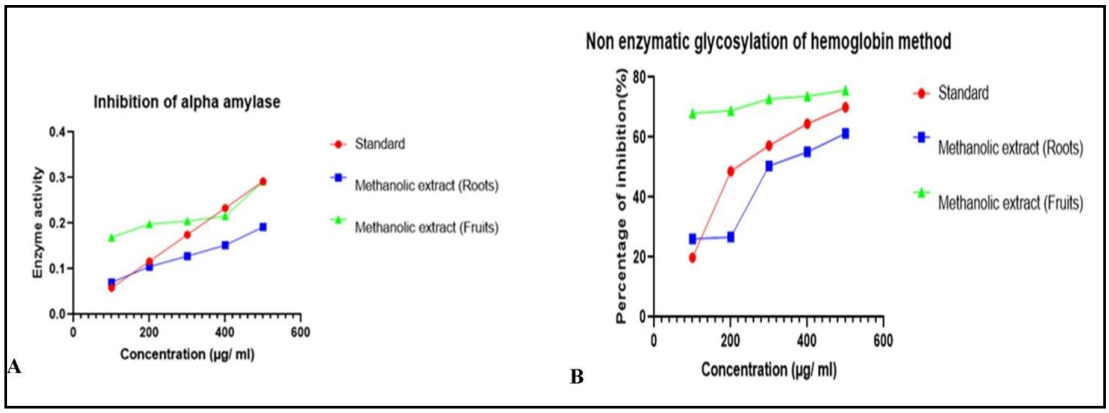

Fig.8: The anti-diabetic potential of methanolic extracts of fruits and rots using alpha amylase inhibition (A) and non enzymatic glycosylation of hemoglobin method (B). In both methods fruit extract showed higher anti-diabetic activity proving promising agent

3.6 Cytoprotective activities:

Cytoprotective activities of both methanolic extracts were carried out using MTT assay in 24 H-HeLA cells. HeLa cells are human epithelial cells from a fatal cervical carcinoma. The cell line was derived from cervical cancer cells taken from Henrietta Lacks, in 1951. Horizontal gene transfer from human papillomavirus 18(HPV18) to human cervical cells created the HeLa genome which is different from either parent genome in various ways including its number of chromosomes. HeLa cells have a modal chromosome number of 82, with 4 copies of chromosome 12 and 3 copies of chromosomes 6, 8, and 17. HeLa cells are adherent cells (they stick to surfaces) and maintain contact inhibition in vitro. The present study reveals no cytotoxicity in the tested concentrations and doesn't affect cell growth/division. May be with higher concentration with different cell lines it may or may not show positive result.

4. Conclusion:

Coccinia grandis a traditional folk medicinal plant had shown ample number of phytochemicals which could be used as potent pharmacological agent. The fruit extract had shown better antibacterial, antioxidant, anti-inflammatory, anti-diabetic and cytoprotective activities as compared to root extract. The concentration of phenolic contents and flavonoids was also found much greater in fruit extract as compared to root extract. So one may consider the fruit extract as potential pharmacological source for the further developmental studies.

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