A study on medicinal properties of *Coccinia grandis*

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Abstract

Coccinia grandis is used as a vegetable in south Asian countries. All parts of this plant is considered to have medicinal properties and are used to treat various diseases *viz.,* skin diseases,jaundice, diabetes etc..The present study clearly established that *Coccinia grandis* is antimicrobial against both gram positive and gram negative bacteriae and also antiamylolytic.

Key wors: antiamylolytic, antibacterial,*Coccinia grandis,* diabetes, jaundice, medicinal plant swarming motility

INTRODUCTION

Diabetes, a chronic, metabolic disease that leads to high blood sugar and over time it leads to damage to various organs such as eyes, kidneys, nerves etc., It occurs in three stages, such as prediabetic, where the level of blood sugar is higher than normal level which cannot be diagnosed. Similarly, Type 1 diabetes is an autoimmune disease and Type 2 diabetes occurs when pancreas produces little or no insulin., Globally 422 million people are diabetic and majority of them are from developing and under developed countries and annual death rate due to diabetes are 1.6 million . Number of new cases and prevalence of the diabetes increases for the past 10 years (WHO 2021).

The *Coccinia grandis* commonly called as Ivy guard, is used as vegetable and grown in South Asia. It is a creeping plant. All the parts of the plant are edible and have got various medicinal uses such as antibruises and anti-itching from insect bites, treatment against cataract, skin diseases such as leprosy, fever, jaundice, mastcell-stabilizing, antianaphylatic and antihistaminic potential, urinary tract infections, respiratory tract infections, ulcerations etc., Similary it is used as an antioxidative, anti-inflammatory and antimicrobial agent(Ramachandran et al., 2014). Very limited information is available on its anti diabetic effect so the present study aimed to analyse the antidiabetic activities *in vitro*. In addition to this its antimicrobial activities were also studied using the two different extracts such as ethanolic and isopropyl alcohol extracts of *Coccinia grandis*.

MATERIALS AND METHODS

Preparation of Plant Extracts

The collected leaf samples were washed air dried and grinded into powder. The powder was suspended in both acetone and isopropyl alcohol solution at a concentration of 20%. The mouth of the test tubes were covered with aluminium foil and was kept at room temperature for 3 days. After that, it was placed on a platform shaker for 1 day. Then the mixture were transferred to tubes and centrifuged for 10 minutes at 4000rpm at 25^0C. The supernatant was collected and dried at 50^0C, the dried powder was mixed with distilled water in eppendorf tube and stored at 4^0C.

Detection of Phytochemicals

Detection of Glycoside: Concentrated Sulphuric acid test

To one ml of the extract one ml of concentrated sulphuric acid was added and allowed to stand for two minutes. A reddish color precipitate indicates the presence of glycosides.

Detection of Phenols: Ferric Chloride Test

To 3-4 drops of ferric chloride solution, add two ml of plant extract was added. Bluish black colour confirms the presence of phenol.

Detection of Terpenoids: Salkowski test

Extract (5ml) was mixed with chloroform (2ml) and concentrated sulphuric acid (3ml) was carefully added to form a layer. A reddish brown coloration formed at

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the interface showed positive result for the presence of terpenoids.

Detection of saponins:Foam test

0.5 ml of plant extract was added to two ml of water and shaken well continuously for 2 minutes. The foam formed after shaking persists for ten minutes. It indicated the presence of saponins

Table: 1. Antimicrobial activity of extracts of *Coccinia grandis*

Plant extracts	Zone of Inhibition			
	Pseudomonas	Klebsiella	B. licheniformes	E. coli
Coccinia grandis - acetone	8mm	6mm	10mm	No clear zone
Coccinia grandis- isopropyl alcohol	No clear zone	9mm	6mm	10mm

Table 2. Swarming Motility of bacteriae in *C.grnadis* extracts

Plant extracts	Swarming Motility			
	Pseudomonas	E. coli	Proteus	B. liccheniformes
Coccinia grandis - acetone	14mm	28mm	10mm	43mm
Coccinia grandis isopropyl alcohol	29mm	25mm	16mm	10mm

Table 3. Minimal Inhibitory Concentration (MIC) - Minimal inhibitory concentration of plant extracts against Escherichia coli

	E. coli	
	Optical density (OD)	
Amount of plant extracts in microliter	*Coccinia grandis* - acetone	*Coccinia grandis*- isopropyl alcohol
50	0.04	0.5
100	0 04	0.12
150	0.05	0. 17
200	0.05	0.16

Anti Amylolytic Activity

Plate Assay:

Enzyme Preparation:

Bacillus culture was inoculated to 100ml of nutrient broth and incubated for 24 hours at 37^{0}C. After incubation the broth was centrifuged at 4000rpm for 10 minutes. The supernatant was collected and stored at 4^{0}C in a sterile test tube.

Plate Assay:

Sterile starch casein agar plates were prepared and 5 'wells were made. One hundred ml of enzyme was loaded into the central well and the surrounding 4 wells were loaded with constant amount of enzyme (100ml) and varying amount of plant extract (25µl, 50µl, 75µl, 100µl). The plates were incubated at 37^{0}C for 24hours. After incubation the zone of clearance was observed by adding iodine solution to the plate.

Antimicrobial activity :-

Nutrient agar plates were prepared and swabbed with 24 hours old culture (*Pseudomonas aeruginosa, Escherichia coli, Bacillus licheniformis, Klebsiella*

Table 4. Detection of phytochemicals in *C.grandis* extracts

Plant extracts	Phenols	Glycosides	Terpenoids	Saponins
Coccinia grandis-acetone	-ve	-ve	-ve	-ve
Coccinia grandis - isopropyl alcohol	+ve	-ve	-ve	-ve

Table 5. Thin layer chromatography of extracts of *C. grandis*

Plant extracts	Solvent (cm)	Solute (cm)	Retention Factor (Distance travelled by solute/Distance travelled by solvent)
Acetone extract of *Coccinia grandis*	9	8.4	8.4/9=0.933
Isopropyl alcohol extract of *Coccinia grandis*	9	5.6	6.22

Pneumonia). One hundred µl of plant extract was added on two wells of nutrient agar plates using sterile micropipette and allowed to diffuse at room temperature and incubated at 37^{0}C for 18-24 hours. A plate without plant extract was set as constant. After incubation the diameter of the zone of clearance was recorded.

Swarming Motility

Nutrient Agar was prepared with 2% agar concentration and 100ml was poured over the petriplate and allowed to solidify. Nutrient Agar was Prepared with concentration of agar 0.75% and dispensed 10ml into a test tube and sterilized separately. After sterilization 100ml of plant extract was added to each 10ml semisolid agar and mixed well. After that it was poured on the solid agar plate, and allowed to solidify. After settling of the plate, 1µl of 24 hours old Culture of *Proteus, Escherichia coli, Pseudomonas aeruginosa, Bacillus licheniformis* were placed on the top of the agar plate and allowed to stand

and incubated at 37^{0}C for 24 hours. After incubation the movement was measured.

Minimal Inhibitory Concentration

One hundred µl of (*Escherichia coli)* culture was added into all the test tubes as fixed volume. Into these tubes different concentrations of plant extract were added. The entire volume of test tube was made up to 5ml with nutrient broth and the optical density was checked at 620nm before incubation and values are noted. All the tubes were incubated at 37^{0}C for 24 hours and after incubation the optical density was noted at 620nm. 0.1ml was taken from the tube and spreaded over the nutrient agar plate and the result was found out after incubation at 37^{0}C for 24 hours.

Thin Layer Chromatography

Ten µl of each extract was loaded on to TLC plates in a narrow band. The bands were eluted using mobile solvent systems. The developed plates were air dried under a stream of fast moving air for 5 days to remove traces of solvent on the plates. The overnight cultures of bacteria which grow in nutrient broth were sprayed on to the TLC plate in the Laminar flow cabinet and the plates were incubated overnight at 35°C and 100% relative humidity in the dark. The chromatograms were dipped in an aqueous solution of 0.8 gL-1 MTT for 15s and incubate at 28 ÚC for 24 h. After overnight incubation, the chromatograms were dipped in 70% ethanol for 10 seconds and the zone of inhibition was observed in the blue background.

RESULTS AND DISCUSSION

Antimicrobial activity:

Acetone extracts of Coccinia grandis

When this extract was used against *Pseudomonas aeruginosa,Klebsiella pneumonia, Bacillus licheniformis and Escheria coli*, the zone of inhibition was 8mm, 6mm,10mm, and no inhibition, respectively. Among hese pathogens *Escherichia coli* showed highest sensitivity against acetone extract of *Coccinia grandis*

Isopropyl alcohol extracts of Coccinia grandis

When this extract was used against P*seudomonas aeruginosa,Klebsiella pneumonia, Bacillus licheniformis and Escheria coli*, the zone of inhibition was no inhibition, 9 mm, 6 mm and 9mm inbibition. respectively. Among these pathogens *Pseudomonas* showed highest sensitivity against isopropyl extract of *Coccinia grandis*

Arulraj (2016) identified the highest antimicrobial activity of acetone extract of *Coccinia grandis* against *K. pneumoniae,* ethyl aceate extract against *Streptococcus pneumoniae* and minimum inhibitory activity from the methanolic and ethyl Acetate extracts against *P. vulgaris and Pseudomonas aeruginosa.*

Syed Zeenat (2009) studied the antimicrobial activity of bioactive compounds of fruits of *Coccinia indica* against pathogenic bacteria and found that highest activity was shown by petroleum ether and methanolic extract and the aqueous extracts didn't have significant activity on the test pathogens and also identified the presence of phytochemicals such as alkaloids, tannins, saponins, flavonoids, glycosides and phenols.

Swarming Motility

Acetone extract of *Coccinia grandis*

The swarming motility of the *Coccinia grandis* extract was tested against *Pseudomonas aeruginosa, Escherichia coli, Proteus and Bacillus licheniformis*, the movement of these organisms against the extract was measured as 14mm, 28mm, 10mm, and 43mm, respectively. Among these pathogens *Bacillus licheniformis* showed highest movement against acetone extracts of *Coccinia grandis.*

Isopropyl alcohol extract of Coccinia grandis

The swarming motility of the *Coccinia grandis* isopropyl alcohol extract was tested against *Pseudomonas aeruginosa, Escherichia coli, Proteus and Bacillus licheniformis,* the movement of these organisms against the extract were measured as 29mm, 25mm, 16mm, and 10mm, respectively. Among these pathogens *Proteus* showed highest movement against isopropyl alcohol extract of *Coccinia grandis*.

Minimal Inhibitory Concentration (MIC)

Minimal inhibitory concentration of plant extracts against Escherichia coli:-

At the zero time in Minimal Inhibitory Concentration the OD remains same whereas after 24 hrs of incubation when the OD of different concentration 25µl, 50µl, 75µl, 100µl, 150ìl and 200ìl were studied there was a gradual increase in the inhibitory effect on each test pathogens were observed, as inferred from the elevation in the OD values. At 25µl to 75µl concentrations of the extract, the inhibition was very less when compared to the concentration of 100µl. Marked increase in the inhibition was observed at 200µl concentration. When MIC results were compared with the zone of inhibition in the agar diffusion method similar results were observed.

Pratoomsoot *et al*., (2020) found that all the extracts of *Coccinia grandis* had antimicrobial antioxidant properties, synergistic properties. Minimal Inhibitory Concentration and Minimal Bactericidal Concentrations values ranged between 0.0625-32 mg\ml and from 0.0625->256 mg\ml. Immanuel

Sagayaraj and Nanditha (2019) found that different parts of *Coccinia grandis* have plenty of the phytochemical compounds such as legnins, tannins, phenols etc., which have highest impact on the pharmacological applications such as treatment against various infectious diseases, antioxidant property, antidiabetic property etc., Sivaraj *et al*., (2011) studied the antibacterial activity of *C.grandis* leaf extract using solvents such as acetone, ethanol, methanol, aqueous and hexane against five bacterial sp. and found that ethanol leaf extract of *C.grandis* showed high antibacterial activity against *S.aureus, B.cereus, E.coli, K.pneumoniae and S.pyogens*. Minimal Inhibitory Concentration of the leaf extract against each test organism showed that the zone of inhibition is directly proportional to the concentration , and the range falls between 31.25µg/ml to 1000µg/ml of the extract.

Phytochemicals:-

Phenol was absent in the acetone extract of *Coccinia grandis* and found present in isopropyl alcohol extract of *Coccinia grandis*. Glycosides were absent in the acetone and isopropyl extracts of *Coccinia grandis.* Terpenoids were absent in acetone isopropyl extracts of *Coccinia grandis*. Saponins were absent in acetone and in isopropyl extracts of *Coccinia grandis.*

Thin Layer Chromatography (TLC)

Movement of solvent was constant (9cm). Movement of acetone extract of *Coccinia grandis* (sample) was 8.4 cm (Rf = 8.4/9=0.933).Movement of isopropyl alcohol extract of *Coccinia grandis* (sample) was 5.6cm (Rf = 5.6/9=0.622). Ren *et al*., (2015) found that the *Murraya koenigii* leaves extract contains flavonoids (confirmed using TLC), which was shown to have antimicrobial activity against human pathogens.

Plate assay:

Different concentrations (25,50,75,100 microliter) of both acetone and isopropyl alcohol extracts of *Coccinia grandis* with fixed concentration of enzyme was mixed and added into the well to study the activity of plant extract on the enzymes and found that the activity of enzyme gradually decreased with the increased concentration of plant extracts and along with that the zone of inhibition also decreased.

Al-Quraishy *et al*. (2015) conducted a review on medication of *diabetes mellitus* and anti-diabetic medicinal plant and found that large number of plants are used for the treatment of diabetes which leads to the development of new drugs. Due to the presence of bioactive compounds which is anti diabetic in nature they may be used in future for the treatment of *diabetes mellitus.*

Petchi *et al*. (2014) identified the anti-diabetic activity of herbal formulation in streptozotocin induced diabetes with a dose level of 250 and 500 mg/kg and evidenced the decreased levels of blood glucose, HbA1c, total cholesterol, triglyceride, low density lipoprotein (LDL) cholesterol, urea, creatinine, SGOT, and SGPT, and increase in plasma insulin, HDL-cholesterol, liver glycogen, and total protein levels.

Kumar *et al*.,(2018) investigated the antioxidant activity and potential inhibition of starch digestive enzymes by the herbal extracts of *Coccinia grandis* leaves and found that it inhibits saliva alpha amylase,pancreatic alpha amylase and yeast alpha glucosidase.

Attanayake *et al*.,(2015) found that the long term effect of aqueous leaf extract of *Coccinia grandis* leads to increased biosynthesis of insulin probably by]"-cell regeneration in the pancreas which confirms the antihyperlipidemic activity of leaf.

Betsy Sunny *et al* ,(2020) studied the antihyperglycemic activity of methanol, ethanol and aqeous extracts of various parts of *Coccinia grandis* plant and found that out of all the extracts used, methanol extract has more antidiabetic property.

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