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Antibacterial and antifungal activity of coccinia grandis instant juice powder

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ABSTRACT

Coccinia grandis L., (Ivy gourd) of the family *Cucurbitaceae* is distributed in tropical Asia, Africa and is commonly found in India, Pakistan and Srilanka. The *Cucurbitaceae* family is commonly known as gourd, melon, and pumpkin family. *Coccinia grandis* plays a major role in the medicinal properties. The main aim of this work is to study the antibacterial, antifungal activity of *coccinia grandis* instant juice powder. *Coccinia grandis* fruits were processed to obtain pulp and made into juice powder. The flavonoid, tannin, phytosterol, glycosides were exist in both aqueous and ethanol extracts of *coccinia grandis* instant juice powder. The antibacterial and antifungal studies revealed a significant activity against bacterial and fungal strains. It showed 2 to 5.1 mm zone of inhibition in the aqueous and solvent extract. Compared with solvent extracts, aqueous extract has more potential. This may be attributed to the presence of phytochemical in *coccinia grandis* instant juice powder. It is concluded from the current study that the phytochemicals of *coccinia grandis* remained active evenafter thermal processing. Hence *coccinia grandis* instant juice powder could be a good source of phytochemical with potential antibacterial and antifungal property.

Keywords: Coccinia grandis L, Cucurbitaceae, flavonoid, tannin, phytosterol, glycosides

ARTICLE INFO

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1. Introduction

Ivy Gourd (Coccinia indica) is extensively used as vegetable and grown wildly throughout Indian subcontinent. It is commonly known as 'Kundru' in India. kovakai in Tamilnadu. Ivy plant has been used in traditional medicine a household remedy for various diseases, including biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders. For the last few decades, some extensive work has been done to establish the biological activities and pharmacological actions of Ivy Gourd and its extracts.Polyprenol is the main yellow bioactive component of Ivy Gourd has been shown to have an antidyslipidemic of biological actions. Anti-inflammatory, antioxidant, antimutagenic, antidiabetic, antibacterial, antiprotozoal, antiulcer, hepatoprotective, expactorants, analgesis, anti inflamatory are the reported pharmacological activities of Ivy Gourd (Goldy yadav et al, 2014). Its leaves and fruits also have antiOxidant property (Ashish K et al., 2011).

Aqueous extract of the leaves shows significant antimalarial activity (Amalesh *et al.*, 2011). Root extracts of *Coccinia grandis* possesses anti-oxidant activity (Preeti Bhadauria *et al.*, 2012). The leaves of this plant show antiulcerogenic and antioxidant effect (Papiya Mitra *et al.*, 2008). The leaf and stem extracts exhibit anti-bacterial activity (Umbreen Farrukh *et al.*, 2008). The object of the present study is to examine antifungal activities and antibacterial activities against some fungi and bacterial strains.

2. Materials and Methods

Collection of raw materials

The fully mature fruits of *coccinia grandis* were collected from market in erode district. The fruits were washed and blanched thoroughly.

Preparation of instant juice powder:

The properly selected and prepared fruits were then passed through juice extractor and the pulp was collected. To the pulp was added preservative (citric acid) and stored. The pulp thus obtained had a brix of 40 degree was concentrated through a refractometer. To the concentrated pulp was next added powder sugar. The mixture was blended together to uniform consistency. The prepared pulp was put in suitable stainless steel trays, forming about 1/2 inch thick layer and placed in cabinet shelf dryer at an initial temperature was next brought up to 70°C and there allowed to dehydrated running overnight. The product, which was in a semi dry state, overturned and allowed in the dehydrator for a further few hours till the desired level of the dehydration, had been attained. The product was scratched from the travs and passed through a suitable grinder. The product was packed under controlled condition in plastic bags. (Saeed et al., 2010)

Extraction Preparation

Aqueous Extract

To obtain the aqueous extracts, about 10 grams of the *Coccinia grandis* instant juice powder were homogenized using 100 ml of water. They were added to soxhlet apparatus and the boiling point of water was set up at 100°C. The water evaporates continuously and was International Journal of Pharmacy and Natural Medicines

recycled, thereby extracting the compounds present in the samples. They were continuously extracted until the solution loses the color.

Acetone Extract

Ten grams of the *Coccinia grandis* instant juice powder were homogenized using 100 ml of acetone. They were added to soxhlet apparatus and the boiling point of acetone was set up at 55°C. The solvent was recycled, thereby extracting the compounds present in the samples. They were continuously extracted until the solution loses the color. The extract was then transferred to a sterile petri dish and kept for evaporation of acetone at room temperature. Residues of extracts were collected and stored in refrigerator.

Ethanol Extract

Ten gram of the *Coccinia grandis* instant juice powder was homogenized using 100 ml of 70% ethanol. They were added to soxhlet apparatus and boiling point of the ethanol was set up at 78°C. The solvent was recycled, thereby extracting the compounds present in the samples. They were continuously extracted until the solution loses the colour. The extract was then transferred to a sterile petri dish and kept for evaporation of ethanol at room temperature. Residues of extracts were collected and stored in refrigerator.

Chloroform Extract

Ten gram of the *Coccinia grandis* instant juice powder was homogenized using 100 ml of 70% ethanol. They were added to soxhlet apparatus and boiling point of the ethanol was set up at 78°C. The solvent was recycled, thereby extracting the compounds present in the samples. They were continuously extracted until the solution loses the colour. The extract was then transferred to a sterile petri dish and kept for evaporation of chloroform at room temperature. Residues of extracts were collected and stored in refrigerator (Poovendran *et al*, 2011).

Phytochemical Screening

Phytochemical screening was done by following method.

Tests for Alkaloids

A small portion of the extract was stirred with a few drops of dilute hydrochloric acid and filtered. The filtrate may be tested carefully with various alkaloid reagents such as:

- Mayer's reagent Cream precipitate
- Dragondroff's reagent Orange brown precipitate
- Hager's reagent -Yellow precipitate
- Wagner's reagent Reddish brown precipitate.

Tests for carbohydrates & glycosides

The minimum amount of extracts were dissolved in 5 ml of distilled water and filtered. The filtrate was subjected to test for glycosides.

Molisch's test:

The filtrate was treated with 2-3 drops of 1% alcoholic alpha naphthol, and 2 ml of concentrated sulphuric acid was added along the sides of the test tube. Violet colour was obtained.

Fehling's test:

The filtrate was treated with 1 ml of Fehling's solution and heated. A reddish orange precipitate was obtained. **Tests for glycosides**

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Another portion of the extract was hydrolyzed with hydrochloric acid for a few hours on a water bath and the hydrolysate was subjected to Legal's, Borntrager's test to detect the presence of different glycosides.

Borntragers's test:

Hydrolysate was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. No colour change in Ammonical layer was observed.

Test for phytosterol

1 g of the extract was dissolved in few drops of dilute acetic acid, 3 ml of acetic anhydride was added followed by few drops of conc. sulphuric acid. Appearance of bluish green colour shows the presence of phytosterol.

Test for fixed oils and fats

Small quantity of the various extracts was separately pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oil.

Test for saponins

The extract was diluted with 20ml of distilled water and it was agitated on a graduated cylinder for 15 min. The presence of saponins was indicated by formation of 1cm layer of foam.

Test for tannins and phenolic compounds

Small quantities of various extracts were taken separately in water and tested for the presence of phenolic compounds and tannins with

- Dilute ferric chloride solution (5%) violet colour
- 1% solution of gelatin containing 10% NaCl white precipitate
- 10% Lead acetate solution white precipitate.

Test for proteins

Dissolved small quantities of various extracts in a few ml of water and treated with

- Million's reagent Red colour
- Ninhydrin reagent Purple colour
- Biuret Test Equal volume of 5% solution and 1% copper sulphate solution were added. Pink or Purple colour.

Test for flavonoids- Shioda's test

To the extract, Magnesium turnings and dew drops of concentrated hydrochloric acid were added and boiled for 5 minutes. Red colour was obtained.

Test for lignin

With alcoholic solution of phloroglucinol and hydrochloric acid - Red colour was observed.

Detection of volatile oils

About 50 g of powered material was taken in a distillation apparatus and subjected to hydrodistillation for the detection of volatile oil (Elamathi and Muhammad, 2012)

Bioactivity of *Coccinia grandis* instant juice powder

The petri dishes were cleaned, dried and sterilized. Then they were filled with a Nutrient Agar medium with uniform thickness. After solidifying, the plates were inoculated with the above mentioned organisms of bacteria and fungi. Three holes were made in the inoculated plates by means of aluminum or stainless steel sterile borer, with the height of 5 cm and 3-5 mm internal diameter. In each hole, Ciproflaxin and Trioderm standard drugs and extracts of each samples were added under aseptic conditions. The International Journal of Pharmacy and Natural Medicines ISSN: 2321-6743

diffusion. All the petri dishes were incubated at 37°C for 24 hours for bacteria and 25°C for 48 hours for fungal strains. The zone of inhibition was measured by using scales. (Reddy et al 2009).

3. Results and Discussion

Physico Chemical Analysis

The *Coccinia grandis* instant juice powder *and* analysed for its physicochemical characters, which were presented in table-1. Acid insoluble ash comprised of 0.89 ± 0.26 , Sulphated ash content was 2.13 ± 1.09 . The total ash and water soluble ash value were 1.45 ± 0.986 and 1.22 ± 0.36 respectively. Further results showed that moisture content and foaming index were found to be 1.66 ± 0.99 and 1.5 ± 0.59 respectively.

Table 1:	Physico chemical analysis in coccinia	grandis
	instant juice powder	

J F - ···						
S.No	Parameters	Values				
	determined	(Mean ± Standard)				
1	Total Ash (g)	1.45±0.986				
2	Acid Insoluble Ash (g)	0.89±0.26				
3	Water solube ash (g)	1.22±0.36				
4	Sulphated Ash (g)	2.13±1.09				
5	Loss on drying (g)	1.66±0.99				
6	Foaming Index (cm)	1.5±0.59				

The phytochemical characteristics were summarized in Table-II. The results revealed the presence of medically active compounds in aqueous extracts, ethanol extracts and acetone extracts of *coccinia grandis* instant juice powder. The presence of flavonoids observed in aqueous, ethanol and acetone extracts.

Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms in vitro. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall (Marjorie *et al* 1996).Flavonoid is effective antioxidant and show strong anticancer activity (Salah *et al*, 1995).

Saponin present only in aqueous extracts of *coccinia grandis* instant juice powder. The aqueous fruit extract revealed to contain saponin which are known to produce inhibitory effect on inflammation (Just *et al*, 1998). Alkoloids present in ethanol extracts of *coccinia grandis* instant juice powder. Alkoloids have been associated with medicinal uses for centuries and one of their common biology properties is their cytotoxicity (Nobori *et al*, 1994). Glycosides exist in all the extracts of *coccinia grandis*

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instant juice powder. Glycosides are known to lower the blood pressure according to many reports (Nyarko *et al*,1990). The presence of tannin, phytosterol, and volatile oils were noted in both aqueous and ethanol extracts. Natural antioxidants mainly come from plants in the form of phenolic compounds such as tannin, phytosterol, and tocopherols, phenolic acids (Ali *et al*, 2008). In aqueous extract lignin, volatile oils, protein were also noted.

The antibacterial and antifungal activity of fresh pulp of *Coccinia grandis* and *coccinia grandis* instant juice powder were examined by preparing aqueous and ethanol extracts in the present study. The results are given in Table-III. The zone of inhibition produced by *coccinia grandis* instant juice powder and fresh paste, were compared with standard drugs such as cephalaxin and Trioderm for antibacterial and antifungal activity respectively. It is evident from the table that there is significant activity against bacterial and fungal strains by the aqueous extract and ethanol extraction. The zone of inhibition of standard antibacterial (Cephalaxin) drugs were ranged from 5-10 mm against the bacterial strains such as E.coli, Staphylococcus and Klebsiella Pneumonia where as its ranged from 2 to 6.1mm in case of

aqueous extract and 2.5 to 5 mm in case of ethanol extract, when compared to solvent extraction, aqueous extract of *coccinia grandis* instant juice powder were more potential against the bacterial strains. The zone of inhibition of standard antifungal (Trioderm) drugs were ranged from 15 to 30 mm against the fungal strains such as Alternaria and Aspergillus niger whereas it range from 3 to 5 mm in case of aqueous extract and 1.5 to 5 mm in case of ethanol extract, when compared to solvent extraction, aqueous extract of *coccinia grandis* instant juice powder were more potential against the fungal strains.

Bhattacharya (2010) evaluated the antifungal activity of the *Coccinia grandis* extract against the Candida albicans-II, Candida tropicalis, Aspergillus Niger, Saccharomyces cerevisiae, Candida tropicalis II, Cryptococcus neoformans and Candida albicans ATCC. Ethanol extract is more significant in producing antifungal activities. Nonpolar fractions in the extract possess a higher level of antifungal properties. Aqueous extract is more sensitive for both strains of Candida albicans and Ethanolic extract is more sensitive for Aspergillus Niger and both strains of Candida albicans.

S.No	Phytoconstituents	Aqueous Extracts	Ethanol Extracts	Acetone Extracts	
1	Alkaloids	-	+	-	
2	Flavonoids	+	+	+	
3	Lignin	+	-	+	
4	Tanin	+	+	-	
5	Saponin	+	-	-	
6	Protien	+	-	-	
7	Phytosterol	+	+	-	
8	Fixed oils and Fats	-	-	-	
9	Glycosides	+	+	+	
10	Volatile oils	+	+	+	

Table 2: Phytochemical analysis in *Coccinia grandis* instant juice powder

(+) indicates presence; (-) indicates absence

Table 3: Anti bacterial and antifungal activity of coccinia grandis instant juice powder

S.No	S.No Micro organism Zone of inhibition in mn					mm	
	Bacterial	Standard		Ethanol		Aqueous	
	organisms	Drug(mm)		extract(mm)		extract(mm)	
		IJP	FP	IJP	FP	IJP	FP
1.	E. coli	10.0	10.0	4.5	5.0	6.1	6.1
2.		10.0	10.0	3.0	3.5	4.5	5.1
3	Staphylococcus	10.0	5.0	2.5	1.0	3.0	0
	Klebsiella						
	Pneumonia						
S.No	Fungal strains	Standard		Ethanol		Aqueous	
		Drug		extraction		Extraction	
		IJP	FP	IJP	FP	IJP	FP
1	A 14	15	20.0	5.0	2.0	2.0	5.0
1.	Alterneria	15	20.0	5.0	3.0	3.0	5.0
2.	Aspergillus Niger	20.0	30.0	0	1.5	0	5.0

IJP- Instant Juice powder, FP- Fresh Puree

4. Conclusion

As far as the physicochemical property is concerned, *Coccinia grandis* istant juice powder contains high total ash content which is attributed to high mineral value. The phytochemical screening is an evident of antibacterial and antioxidant property of *Coccinia grandis* instant juice powder. It is concluded from the current study that the phytochemicals of *Coccinia grandis* remained active evenafter thermal processing. Hence *Coccinia grandis* instant juice powder could be a good source of phytochemical with potential antibacterial and antifungal property.

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