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Phytochemical compound from *Coccinia indica,* its relevance to antimicrobial and anti-inflammatory activities

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Abstract : *The present study attempts to evaluate the phytochemical constituent of aqueous, ethanol and methanol extract of leaves of Coccinia indica. The phytochemical screening which involves both the qualitative and quantitative analysis revealed either the presence or absence of secondary metabolites; Alkaloid ,Tannin, Saponin, Protein, Phenol, Terpenoid, Flavonoid in all the three extracts of C.indica.*

The leaf extract of this plant shows antimicrobial activities against common pathogen by agar-well diffusion method. The sample extract were used against Staphylococcus spp and Aspergillus spp. Methanolic extract of C.indica leaves showed zone of inhibition.

In the leaf extract of C.indica anti-inflammatory property have also been noticed. During this study the presence of heavy metals were noticed in small quantity.

Keyword*: Coccinia indica, Alkaloid, Tannin, Flavonoid, Phenol.*

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Introduction :

There are hundreds of medicinal plants that have a long history of curative properties against various diseases and ailments. However, screening of plant for their activity is very essential and needs urgent attention in order to know the value of the plant. (Syed et.al. 2009)

Coccinia indica belongs to the family cucurbitaceae, commonly called little gourd. It is indigenous to Bengal and other parts of India. The plant has been used extensively in ayurvedic and unani practice in the Indian subcontinent. (Khadabadi and Deokate 2012). It is an annual creeper which is found spreading on ground and twining around the trees. Leaves are triangular or pentagonal in shape.

Leaves of this plant are used in Indian folk medicine for treatment of a number of ailments including diabetes, wounds, ulcers, inflammation in eruption of skin, fever, asthma and cough.

In this plant several phytochemicals are present which are beneficial for living organisms. This paper presents the phytochemical and anti-inflammatory properties of leaves of *C.indica*.

Materials and Methods :

Phytochemicals Screening

Sample Preparation:

The fresh leaves of *C.indica* were collected from the campus of Patna Women's College.

The leaves were collected in bulk and dried at room temperature for two weeks. The dried plant materials were grinded with a grinder. The coarse powder was stored in air dried container. From each plant 5gm of powder was extracted with 100ml of ethanol, methanol, and distilled water. The extracts were filtered and used as supernatants (Hagerman et.al.1997).

The methanolic, ethanolic and aqueous extracts of powdered leaves of *Coccinia indica* were subjected to preliminary testing for the detection of major chemical groups. The details of the tests are as follows:

Tannins: 2-3 ml of extract was taken in test tube separately and 1ml of 5% ferric chloride was added to it. Greenish grey coloration of the solution indicated the presence of tannins in the sample (Harbone et.al.1998).

Alkaloid: 1.36 gm of mercuric chloride and 5.00 gm of potassium iodide were taken and dissolved in 100ml of water (Mayer's reagent). Then dil.Hcl and Mayer's reagent were mixed followed by leaf extract. Formation of precipitate show of the presence of alkaloid in it.

Saponin: Two ml of extract was vigorously shaken with 5ml of distilled water in a test tube, allowed to stand for a while at room temperature. Persistent frothing indicated the presence of saponin (Ibrahim et.al. 2014).

Flavonoid: 2-3ml of extract of leaves was taken in a test tube and 1ml of alcoholic ferric chloride was added to it. Dark blue coloration of the solution indicated the presence of flavonoids.

Protein: 2ml of extract was treated with few drops of concentrated nitric acid. Formation of greenish yellow colour indicated the presence of protein.

Terpenoid: 1ml of the extract was taken in a test tube and 1ml of chloroform, 2-3ml of acetic anhydride and 1-2 drops of concentrated sulphuric acid were added. Dark pink or red coloration of the solution indicated the presence of terpenoid.

Phenols: 2-3ml of the extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish colour indicated the presence of phenols.

Five hundred mg of powdered leaves was weighed and put into 100ml plastic bottle. Fifty ml of distilled water was added and shaken for 1 hr. in shaker incubator. This was filtered into 5ml conical flask. Then 5 ml of the filtrate was pipetted out into a test tube and mixed with 3ml of 0.2 M Ferric chloride in 0.1 N HCl and 0.008M Potassium Ferrocyanide. The absorbance was measured with spectrophotometer at 530 nm wavelength within 10 min. A blank sample was measured at same wavelength following Van Burden and Robinson (1981).

500mg of sample was dissolved in 50ml ethanol and filtered. The extract was centrifuge at 3000 rpm for 10 min.

0.5 ml of extract solution was taken and 0.2 ml Folin Ciocalteau reagent was added to it and 10ml

distilled water was also added. The solution was incubated at 27^0 for 30 min. Absorbance was taken at 765 nm using UV Spectrophotometer. Concentration was calculated using galic acid as a standard.

500mg of powdered leaves was weighed and 10ml acetate buffer was added, crushed and centrifuged at 2500 rpm for 10 minute and then filtered.

1ml of the filtrate was added to 5ml alkaline solution (0.1N NaOH+2% Sodium Carbonate+1% Copper Sulphate +2% Sodium Potassium Tartarate) and left undisturbed for 10 minutes. 0.5 ml of FolinCiocalteau reagent was added. The absorbance was taken with spectrophotometer at 750 nm.

Glass wares were washed with tap water and then air dried. Petri plate and Vials were wrapped with almunium foil and kept in wire basket and autoclaved at 15 lbs\inch^2(121^0C) for 15 min. Pipette was kept in pipette can and left in hot air oven at 180^0C for 4 hours.

Potato dextrose agar and nutrient agar were used for preparing the media adopted Aneja (2010) was followed for the preparation of media.

With the help of serial dilution method a dilution of master suspension at 10^{-5} was prepared. For serial dilution of pure culture, 0.1gm pure culture of *Aspergillus* and *Staphylococcus* was taken from loop and mixed in 9.9 ml sterilized saline water in test tube. These test tubes were shaken gently on vortex shaker until the spores were homogeneously diluted in saline water. The dilution was made upto 10^{-5} level. After that from the last tube, i.e 10^{-5}, 1ml suspension were taken were spreaded homogenously on the PDA or NA.

Coccinia indica were collected and dried in sunlight, coarsely powdered and mixed with methanol and filtered with muslin cloth.

Blank disc were sterilized by autoclaving. Sterilized discs were individually impregnated with extracts. Disc were then placed into petriplate containing suspension. The plates were inverted and incubated at 26^0C for 24 hrs. Then antimicrobial activities were determined by measuring diameter of zone of inhibition.

C.indica leaves were collected leaves, washed and dried. They were finely powdered with the help of mortar pestle. 95% ethanol was added and then filtered with the help of muslin cloth. Dilution of extract and standard (diclofenac sodium) were prepared in the concentration range of 10, 20, 30, 40, 60, 80 and 100µg/ml.

0.2 ml of egg albumen, 2.8ml of phosphate buffer saline (PBS, pH-6.4) and 2ml of extract of varying concentration added together. Standard were mixed with similar volume of distilled water, which was taken as control. Then the mixture were incubated at 37^0C for 15 min. and then heated at 70^0C for 5 minutes. After cooling the absorbance was measured at 660nm.

1gm of *C.indica* leaves were cut into pieces. 20ml concentrated sulphuric acid and 10 ml concentrated nitric acid were mixed with the leaves. The solution was heated at 80^0C for 3 hrs. The volume was made upto 100ml by adding distilled water is volumetric flask. Absorbance was taken by using Atomic Absorption Spectrophotometer (AAS). The analysis was done in triplicate.

Results and Discussion :

Phytochemical screening revealed the presence of tannin, alkaloid, saponin, protein, phenol, terpenoid (Table 1 and 2). The tannin, as an anti-diabetic, may contribute to the effects such as antimicrobial, antipyretic, anti-inflammatory, antispasmodic and expectorant activities. Tannin as astringent, properties, hastens the healing of wounds and inflammed mucous membrane.

Table 1. Phytochemical screening of components found in extracts of *C.indica.*

Test	Ethanol	Methanol	Aqueous
Tannin	-ve	+ve	+ve
Alkaloid	+ve	-ve	+ve
Saponin	+ve	+ve	+ve
Flavonoid	-ve	-ve	-ve
Protein	-ve	+ve	+ve
Phenol	-ve	+ve	+ve
Terpenoid	+ve	-ve	-ve

Table 2. spectrophotometric result of phytochemicals (µg/ml)

Test	Concentration (ug/ml)	Absorbance (nm)
Tannin	2.8	0.424 at530nm
Phenol	2.4	0.093 at765nm
Protein	4.8	1.433 at750nm

Methanolic extract of *C.indica* leaves showed zone of inhibition when tested against "***Staphylococcus." 2mm*** respectively, and against "***Aspergillus,"* 3mm** (Fig. 1 and 2).

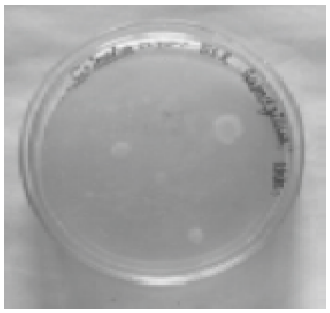

Fig. 1. *C. indica* leaves (3mm) against *Aspergillus*

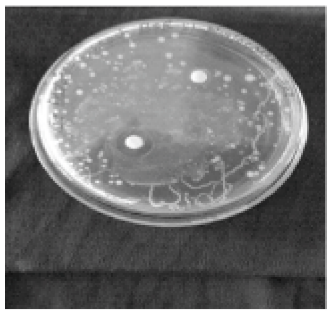

Fig. 2. C.indica* leaves (2mm) Against *Staphylococcus

The anti-inflammatory activity was found to increase with increase in concentration of *C.Indica* (Fig. 3).

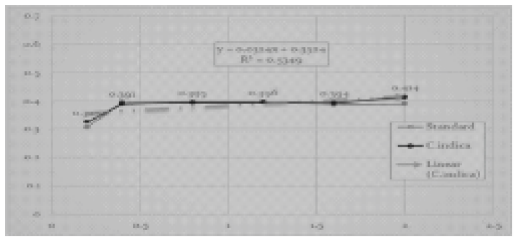

Fig. 3. Graph showing anti-inflammatory absorbance in *C.indica*. (absorbance vs. concentration)

Levels of heavy metals (cadmium, zinc, copper) in *C.indica* were as follows:

1) Zinc – 0.218± 0.2 ppm
2) Copper – 0.131±0.42 ppm
3) Cadmium – 0.009± 0.31 ppm

Conclusion:

The study, has revealed the phytochemical content of *C.indica* leaves. *C.indica* is famous as marvel plant with immense anti-diabetic potential due to presence of terpenoid because it is found to be responsible for ant diabetic activity. It possesses anti-inflammatory, analgesic and anti-microbial properties which is helpful for human welfare.

Therefore, *C.indica* is used in traditional medicine as well as it has various therapeutic properties.

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