Original Research Article

Evaluation of standardisation parameters, pharmacognostic study, preliminary phytochemical screening and in vitro antidiabetic activity of *Coccinia indica* fruits as per WHO guidelines

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ABSTRACT

*Coccinia indica* an annual creeper is available all over India and well known for its antidiabetic property. In the present investigation, aqueous extract, and ethanolic extract of the fruits were made using hot extraction procedure using soxhlet apparatus, decoction and maceration. The qualitative phyto-chemical screening procedure was performed on each extract. Phyto-chemical study reveals that carbohydrates, tannins, phenols, alkaloids, saponins was present in both the extracts. An attempt has been made to highlight this folk herbal medicine through present study which will assist in the identification of fresh as well as dried crude samples of fruits anatomically and physiochemically. TLC finger printing and fluorescence analysis of powdered fruits has been conducted and reported. The antidiabetic activity is conducted by enzyme inhibition (α-glycosidase) in vitro method on each extract and ethanolic extract showed significant inhibition.

Introduction

Now-a-days there is a renewed interest in drugs of natural origin simply because they are considered as green medicine and green medicine is always supposed to be safe. Another factor which emphasizes this attention is the incidences of harmful nature of synthetic drugs which are regarded as harmful to human beings and environment. The advantage of natural drugs is their easy availability, economic and less or no side effects but the disadvantage is that they are the victims of adulteration. The more effective the natural drug more is its demand and the chances of non-availability increases. To meet the growing demand, the natural drug is easily adulterated with low grade material. Adulteration or substitution is nothing but replacement of original plant with another plant material or intentionally adding any foreign substance to increase the weight or potency of the product or to decrease its cost. Therapeutic efficacy of medicinal plants depends upon the quality and quantity of chemical constituents. The misuse of herbal medicine or natural products starts with wrong identification[1]. *Coccinia indicia* a herb that belongs to Cucurbitaceae family and that grows abundantly in India, has been widely used in traditional treatment of diabetes. The plant has also been used extensively in Ayurvedic and Unani practice in the Indian subcontinent (Wealth of India, 1992). *Coccinia indicia* is a good wound healer and reduces any kind of inflammation occurring in body. Herbal substances are new and scientifically well- to lower blood sugar and reduce the late complications of diabetes mellitus[2].

Plant profile - *Coccinia indicia*

*Coccinia grandis*, the ivy gourd, also known as baby watermelon, little gourd, gentleman's toes, tindora or sometimes inaccurately identified as gherkin, is a tropical vine. It is also known as *Cephalandra indica* and *Coccinia indica* [3]
Synonyms
Bryonia grandis, Coccinia cordifolia, Coccinia indica
Calabacita, Calabaza Hiedra, Coccinia grandis, Courge
Écarlate, Kovai, Little Gourd, Tela Kucha, Tindola

Vernacular Names [4]
English: Scarlet-fruited gourd, tindora, kovai fruit
Chinese: Hong gua
Danish: skariagenagurk
Hindi: parval, tindora (tindori or tindola), tinda, tendus, kundru, kunduzi
Japanese: yasai karasuuri
Malay: pepasan, papasan, kovai, kovakka
Spanish: pepino cimarrón
Ayurveda: Bimbi, Kunduru, Raktaphala, Piluparni

Geographic spread
Coccinia grandis native range extends from Africa to Asia, including India, the Philippines, China, Indonesia, Malaysia, Thailand, Vietnam, eastern Papua New Guinea, and the Northern Territories, Australia. Its documented introduced range includes the Federated States of Micronesia, Fiji, Guam, Saipan, Hawaii, the Marshall Islands, Samoa, Tonga, and Vanuatu.

Table 1: Chemical constituents of plant Coccinia indica[7]

| Plant part | Constituent reported |
|------------|----------------------|
| Roots      | Triterpenoid, saponin coccinioside – k(i). C_{41}H_{66}O_{12}  
Flavonoid glycoside ombuin 3-o- arabinofuranoside  
3- o- β- (α-l- arabinopyranosyl)-(1→2) –β-d-glucopyranosyl- (1→3)- β- hydroxyl up – 20(29)- en-28- oic acid  
Lupeol, β-amyrin, and β- sitosterol  
Stigmast -7- en-3-one, |
| Fruits     | Taraxerone, taraxerol, and (24R)-24- ethylcholest- 5- en- 3β- ol glucoside  
B- carotene, lycopene, cryptoxanthin, and apo- 6'- lycopenal  
B- sitosterol and taraxerol |
| Aerial parts| Heptacosane  
Cephalandrol, C_{29}H_{58}O tritriacontane C_{33}H_{68}  
B- sitosterol alkaloids Cephalandrine a and Cephalandrine b. |
| Whole plant| Aspartic acid, Glutamic Acid, Asparagine, Tyrosine, Histidine, Phenylalanine And Threonine Valine Arginine |

Medicinal use of various parts of Ivy Gourd [5-6]

Leaves
- Skin diseases (ring worm, psoriasis itch, sores, pityriasis), skin eruptions of small pox, small lesions of scabies.
- Chronic sinuses.
- Causes cooling effect to eyes.
- Gastro-intestinal disturbance and diseases.
- Alleviate body heat by inducing perspiration in fever.

Stem
- Antispasmodic effect.
- Expectorant.
- Useful in Asthma and bronchitis.

Fruit
- Cures sores on tongue.
- Raw fruit used as vegetable.
- Cure eczema
- Tincture is used internally in gonorrhoea. Cure diabetes and intermittent glycosuria
Root

- Remove pain in joints.
- Aphthous ulcers.

Material and methods

Collection and authentication
*Coccinia indica* L. fruits were collected from local market of Nalgonda, Nalgonda Dist., Telangana and India. All plant materials were collected from the months of December to January 2013-14. The plant material was identified and authenticated by Mr. Siddulu lecturer and head of botany department, Nagarjuna Government degree college, Nalgonda, India.

Chemicals and Reagents
All reagents of analytical grade and highly pure. Enzymes are procured from Synkromax Biotech Pvt. Ltd, Chennai, India.

Equipment requirement
UV Fluorescence Analysis cabinet (SecorIndia/Mumbai), Heating mantle (Sun Bim, India), Rotary Vacuum evaporator (Indosati, India), pH Meter (Elico Ltd/Model no. LI 120, Hyderabad), Pipettes & burettes (Borosil) has been used.

Morphological study
The drug was evaluated by its colour, odour, taste, size, shape and special features, like texture, touch, etc. evaluation was carried based on the morphological and sensory profiles of whole drug.

UV Fluorescence analysis
Powdered fruits of *Coccinia indica* were subjected to analysis under ultra violet light after treatment with various chemical and organic reagents. Three parameters were taken into account i.e., observation under long wave length U.V (365 nm), short wave length U.V (254 nm) and normal day light[8].

| Reagents | Long wave length | Short wave length | Day light |
|----------|------------------|-------------------|-----------|
|          | 0min  | 30min | 24hrs | 0min | 30min | 24hrs | 0min | 30min | 24hrs |
| Con.HSO₄ | Black | Black | Black | Green | Green | Green | Brown | Light yellow | Yellow |
| Con.HCL  | Black | Black | Black | Light Green | Light Green | Green | Light Brown | Brown | Violet |
| Pet.ether | Black | Light yellow | Black | Green | Light Green | Green | Light Brown | Light Brown | Yellow |
| Ethanol  | Black | Black | Black | Light Green | Light Green | Green | Light Brown | Light Brown | Violet |
| Methanol | Black | Light Green | Black | Light yellow | Green | Light Brown | Light Brown | Yellow |
| FeCl₃    | Black | Black | Black | Green | Green | Green | Brown | Brown | Brown |
| Benedicts | Black | Light Green | Black | Light Green | Green | Green | Light Green | Green | Yellow |
| Biuret   | Black | Black | Black | Light Green | Green | Green | Light Brown | Light yellow | Yellow |
| Dragendroffs | Black | Black | Black | Light Green | Light Green | Green | Light Brown | Light yellow | Yellow |
| Mayers   | Black | Black | Black | Brown | Light Green | Green | Light Brown | Light Green | Violet |
| Ninhydride | Black | Black | Black | Light Green | Green | Green | Light Brown | Light Green | Yellow |
| Hagers   | Black | Black | Black | light Green | Green | Green | Light yellow | Yellow | Blue |
Microscopic study [9]

Fig 2: Microscopic characters of *Coccinia indica* fruit

**Proximate analysis** Proximate analysis was carried out for the fruit powder of *Coccinia indica* [10-12]
Table 3: Standardisation parameters of *Coccinia indica* fruit powder

| S.No. | Parameters                          | %w/w   |
|-------|-------------------------------------|--------|
| 1     | Total ash value                     | 9.5%   |
| 2     | Acid insoluble ash                  | 9%     |
| 3     | Water-soluble ash                   | 3.5%   |
| 4     | Alcohol soluble extractive          | 9.6%   |
| 5     | Water soluble extractive            | 34.4%  |
| 6     | Moisture content                    | 20%    |
| 7     | Swelling index                      | 8ml    |
| 8     | Foaming index                       | 111.1  |
| 9     | Foreign matter                      | No foreign matter |
| 10    | True and bulk density               | 0.19g/ml |
|       |                                     | 0.25g/ml |
| 11    | pH                                  |        |
| 1     | Decoction                           | 5.55   |
| 2     | Soxhletation                        | 3.56   |
| 3     | Water maceration                    | 5.86   |
| 4     | Alcohol maceration                  | 3.5    |

**Limit test for iron**

There has been not much intense colour observed than the standard iron solution hence the test passes for the iron[13].

**Extraction**

Extraction, as the term is used pharmaceutically, involves the separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedures[14].

a) **Decoction**

In this process, the crude drug (20g) is boiled in a specified volume of water for a defined time; it is then cooled and strained or filtered. This procedure is suitable for extracting water-soluble, heat-stable constituents. The starting ratio of crude drug to water (320ml) is fixed, e.g. 1:4 or 1:16; the volume is then brought down to one-fourth its original volume by boiling during the extraction procedure[15].

b) **Maceration**

1) **Alcohol maceration**

In this process the drug (20g) is placed with the whole of the alcohol (360ml) in a closed vessel for 7 days. During this period shaking is done occasionally. After 7 days the liquid is strained and marc is pressed.

2) **Water maceration**: This method is similar to alcohol maceration where water is used instead of alcohol[16].

c) **Continuous hot percolation process /soxhlet extraction**

The drug (25g) to be is packed in a paper cylinder made from a filter paper and it is placed in the body of soxhlet extractor. The alcohol (200ml) is placed in the flask. The apparatus is fitted. The process of filling and emptying of the extractor is repeated until the drug is exhausted[16].

Table 4: % yield of different extraction methods

| S. No | Type of extraction             | % yield (w/w) |
|-------|--------------------------------|---------------|
| 1     | Decoction (water)              | 55.4%         |
| 2     | Alcohol maceration             | 28.2%         |
| 3     | Water maceration               | 31.15%        |
| 4     | Soxhlet extraction (Ethanol)   | 37.08%        |

**Preliminary Phytochemical screening**: After extractions the extracts were subjected to a vacuum rotary evaporator and concentrated extracts were obtained along with solvent recovery[17].
## Table 5: Phytochemical screening of different extracts of Coccinia indica fruit powder

| Tests                          | Decoction | Alcohol maceration | Water maceration | Soxhlet extraction |
|-------------------------------|-----------|--------------------|------------------|-------------------|
| Carbohydrates                 |           |                    |                  |                   |
| Molisch test                  | +         | +                  | +                | +                 |
| Fehling’s test                | +         | +                  | +                | +                 |
| Barfoeds test                 | _         | _                  | _                | +                 |
| Benedict’s test               | _         | _                  | _                | _                 |
| Test for pentose’s            | _         | _                  | _                | _                 |
| Tannins                       |           |                    |                  |                   |
| Ferric chloride test          | +         | +                  | +                | +                 |
| Chlorogenic acid test         | _         | _                  | _                | _                 |
| Gelatin test                  | +         | +                  | +                | +                 |
| Saponins                      |           |                    |                  |                   |
| Froth formation test          | +         | +                  | +                | +                 |
| Flavonoids                    |           |                    |                  |                   |
| Alkaline reagent test         | _         | _                  | _                | _                 |
| steroids and triterpenoids    |           |                    |                  |                   |
| Liebermann bur chard test     | _         | _                  | _                | _                 |
| Salkowski test                | _         | _                  | _                | _                 |
| Sulphur powder test           | +         | +                  | +                | +                 |
| test                          |           |                    |                  |                   |
| starch                        | _         | _                  | _                | _                 |
| Proteins                      |           |                    |                  |                   |
| Biuret test                   | _         | _                  | _                | _                 |
| Ninhydrine test               | _         | _                  | _                | _                 |
| Alkaloids                     |           |                    |                  |                   |
| Test for vitamins [18] |
|-----------------------|

**Table 6: Test for vitamins to Coccinia indica fruit extracts**

| Type of vitamin | Decoction | Alcohol maceration | Water maceration | soxhlet extraction |
|-----------------|-----------|--------------------|------------------|--------------------|
| Vitamin D       | +         | +                  | +                | +                  |
| Vitamin A       | –         | –                  | –                | –                  |
| Vitamin C       | –         | –                  | –                | –                  |
| Vitamin E       | +         | +                  | +                | +                  |
| Vitamin K       | –         | –                  | –                | –                  |
| Thiamine        | +         | +                  | +                | +                  |
| Riboflavin      | +         | +                  | +                | +                  |
| Pantothenic acid| +         | +                  | +                | +                  |
| Niacin          | +         | +                  | +                | +                  |

+ = indicates present  
- = indicates absent

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**Saponins**

For detection of saponins required amount of the extract is dissolved in n-butanol and sample is applied on the plates using chloroform: GAA: methanol: water (64:32:12:8) as mobile phase using anisaldehyde sulphuric acid reagent as visualising agent.

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**Phenols**

For detection of Phenols required amount of the extract is dissolved in ether and sample is applied on the plates using acetic acid: chloroform (1:9) as mobile phase using vanillin-HCL as visualising agent.
Alagar Raja et al. / Indian J. Pharm. Biol. Res., 2014; 2(3):54-64

Alkaloids
For detection of Alkaloids required amount of the extract is dissolved in chloroform and sample is applied on the plates using toluene: ethyl acetate: diethyl amine (7:2:1) as mobile phase using dragendroffs reagent as visualising agent.

Tannins
For detection of Tannins required amount of the extract is dissolved in chloroform and sample is applied on the plates using chloroform: ethyl acetate: GAA(6:4:4) as mobile phase using vanilin-H\textsubscript{2}SO\textsubscript{4} as visualising agent.

Carbohydrates
For detection of Carbohydrates of the extract is dissolved in chloroform and sample is applied on the plates using n-butanol: GAA:ether : water (9:6:3:1) as mobile phase. The observed spots are observed under long wavelength and short wavelength for fluorescence and resolved.

| Type of compound | the | RF value |
|------------------|-----|----------|
|                  |     | Decoction | Water maceration | soxhelation | Alcohol maceration |
| Carbohydrates    |     | 0.6       | 0.4              | 0.97        | 0.76              |
| Tannins          |     | 0.76      | 0.51             | 0.97        | 0.95              |
| Phenols          |     | 0.8       | 0.84             | 0.86        | 0.9               |
| Saponins         |     | 0.35      | 0.47             | 0.44        | 0.42              |
| Alkaloids        |     | 0.88      | 0.73             | 0.25        | 0.9               |

Blue colour indicates long wavelength, Green colour indicate short wavelength

Fig 3: TLC finger printing of different extracts of *Coccinia indica* fruits to estimate different phytoconstituents

*In vitro* methods employed in Antidiabetic studies

**Inhibition of alpha-glycosidase enzyme [21]**

The inhibitory activity was determined by incubating a solution of starch substrate (2 % w/v maltose or sucrose) 1 ml with 0.2 M Tris buffer pH 8.0 and various plant extract for 5 min at 37°C. The reaction was initiated by adding 1 ml of alpha-glucoisidase enzyme (1U/ml) to it followed by incubation for 40 min at 35°C. Then the reaction was terminated by the addition of 2 ml of 6N HCl. Then the intensity of the colour was measured at 540nm.
Fig 4: Schematic representation for a) breakdown of oligosaccharides to simple sugars by intestinal amylase b) blocking of intestinal amylase by herbal extract (competitive inhibitor) preventing breakdown of oligosaccharides to simple sugars

Calculation of Percentage inhibition (I %)
Percentage inhibition (I %) was calculated by
\[
I \% = \frac{(A_c - A_s)}{A_c} \times 100
\]
where \( A_c \) is the absorbance of the control and \( A_s \) is the absorbance of the sample

Table 8: % Inhibition of alpha-glycosidase enzyme by coccinia fruit extract

| S.No | Type of extract          | % Inhibition |
|------|--------------------------|--------------|
| 1    | Water Maceration         | 40.50%       |
| 2    | Decoction (water)        | 51.89%       |
| 3    | Soxhlation (ethanol)     | 68.35%       |
| 4    | Alcohol Maceration       | 70.88%       |

Table 9: % In vitro Inhibition of alpha-glucosidase enzyme by alcohol maceration extract in dose dependent manner

| S.No | Concentration of Sample (µg/ml) | % Inhibition |
|------|---------------------------------|--------------|
| 1    | 2                               | 18.98%       |
| 2    | 4                               | 30.37%       |
| 3    | 6                               | 44.83%       |
| 4    | 8                               | 59.4%        |
| 5    | 10                              | 70.88%       |

Fig 5: Dose dependent curve of alcohol macerated extract on enzyme inhibition
Alagar Raja et al. / Indian J. Pharm. Biol. Res., 2014; 2(3):54-64

Discussion

According to the World Health Organization (WHO, 1998), the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and the degree of purity of such materials and should be carried out before any tests are undertaken. Macroscopic studies based on colour of fruits of plant *Coccinia indica* L. a pepo, ovoid, glabrous, greenish brown to yellowish –brown with white linings; no odour and taste. In present study all the parameters are evaluated successfully as per ayurveda pharmacopeia. Fluorescent studies of the fruits powder to wide range of colour changes at day light, UV-chamber (254nm and 365nm). The different extracts of the plant were subjected to the preliminary tests showed the presence of various constituents like Carbohydrates, saponins, tannins, phenols and alkaloids. The present finding reveals that *Coccinia indica* fruits efficiently inhibits alpha-glycosidase enzyme invitro. The alcohol macerated extract showed high inhibition and the range of inhibition ranges from 70.8- 19.84%. The inhibition for decoction is 51.89%, water maceration 40.50%, soxehlated 68.35%. The antiidiabetic action of *Coccinia indica* can also be attributed due to the presence of saponins on alphaglucosidase inhibitory activity.

Conclusion

Diabetes is a serious metabolic disorder. Differences in social structure, psychic stress, obesity, hormonal imbalance and heredity are optimizing the growth of pandemic. *Coccinia indica* is famous plant for its safe anti diabetic property. *Coccinia indica* has been reported earlier but none of the literatures or paper shows the anti diabetic activity of fruit of the above plant as in crude extract. These data show that a reliable, cost saving therapy with traditionally used plants could be a possibility to lower the problems of untreated diabetes because of a lack of synthetic drugs *Coccinia indica* fruit powder extracts has been conducted *in vitro* anti diabetic activity The plant showed significant inhibition activity, so further the compound isolation, purification and characterization which is responsible for inhibiting activity, has to be done for the usage of anti diabetic agent.

Conflict of interest statement

We declare that we have no conflict of interest.

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