Original Article

Phytochemical Screening and GC-MS Analysis in Wild Variety of *Coccinia indica* – An Future Promising Therapeutic Source

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ABSTRACT

Objective: In current, attention has been reverting back to plants as sources of therapeutic agents due to their superior properties. The literature review exposed that there is no report on phytochemical constituents of wild variety of *Coccinia indica*. As best of our knowledge there is no such report on study on the fruits of wild variety of *Coccinia indica.*

Methods: The phytochemical analysis of alcoholic extract of the fruits of wild variety of *Coccinia indica* was done first time. Further study has been preceded using Gas Chromatography – Mass Spectrometry (GC-MS) from alcoholic extract of wild variety of *Coccinia indica* fruit.

Result: Alcoholic extract of wild variety of *Coccinia indica* fruit responded optimistically for Steroids, Terpenoids, Saponins, Flavonoids Alkaloids, Tannins, Glycosides, Phenol and Mucilage during qualitative phytochemical analysis. With reference to Library of Terpenoids, it is confirmed that the following compounds are belongs to the group of Terpenoids and identified Terpineol which is also belongs to the Terpinoid group.

Conculsion: Further investigation may lead to isolation of bio-active compounds and their structural elucidation and screening of pharmacological activity will be cooperative for further drug development.

Keywords: *Coccinia indica,* Wild type, Phytochemical, Bio-active compounds, Gas Chromatography – Mass Spectrometry and pharmacological

INTRODUCTION

Most of the modern researches on herbal medicine have hinged around traditional folklore medicine. The modern medicine has brought with it an array of drugs, none of which is non-toxic and quite safer for human consumption. There are hundreds of medicinal plants that have a long history of curative properties against various diseases and ailments. However, screening of plants for their activity is very essential and needs urgent attention in order to know the value of the plant.

Plants have always been an exemplary source of drugs and many drugs currently available have been derived directly or indirectly from them. Indian system of traditional knowledge *i.e.* Ayurvedic is well known for its effective herbal treatments. There are about 7000 plant species are found in India. Although most of them have a long history in folk medicine, there is lack of written data on their efficacy and safety, especially from human studies.

One such medicinal plant is **Ivy Gourd** (Syn. *Coccinia indica Wight & Arnott*.) is indigenous plant of Central Africa, India and Asia. ***Coccinia indica*** **(Fig. 1)** is belongs to the Family Cucurbitaceae. It is found in climate that is warm and humid. It is found in whole India in wild. It is cultivated abundantly in India (Assam. Bihar, Orissa, Maharashtra, Andhra Pradesh, Tamil Nadu) as a vegetable and its wild form is also found in many parts of India. This plant has been widely used in traditional Indian medicinal system[1].The phytochemistry and pharmacology of one popular anti-diabetic and hypoglycemic plant *Coccinia indica.*

The present study focused to reveal phytochemical constituents from the alcoholic extraction of wild variety *Coccinia indica* fruit and further investigation carried out on Gas Chromatography-Mass Spectrometry

METHODOLOGY

Sample Collection

Wild variety type of *Coccinia indica* (Wight and Arn) Family *Cucurbitaceae* fruits were collected from Alamadhiis geographically located at latitude 13° 12′0" and longitude 13° 12′0", Chennai, TamilNadu, India during the month of January 2015. Two varieties of *C. indica* are recognized; tender fruits are bitter in one variety and not bitter in another, and the latter is used in Asian cooking[2-4]. Morphologically no difference is evident between them, however; both varieties are invasive and are found to grow close to each other.

Processing of Sample

Mature unripe fruits were washed with running tap water and drained. The fruits were sliced then shade dried in room temperature for 10 days and pulverized in mechanical grinder into coarse powder and stored in airtight containers for further process. The extract was prepared using alcohol by Soxhlet apparatus. As per the protocol reported in[5]was followed to obtain *Coccinia indica* mucilage extract.

Phytochemical Screening

Phytochemicals are the natural bioactive compounds found in plants. The qualitative analysis of phytochemicals such as Steroids, Terpenoids, Saponins, Flavonoids, Alkaloids, Carbohydrates, Tannins, Glycosides, Phenol and Mucilagewere performed in the study. The Phytochemical analyses were carried out as described by[6] to screen the presence of the bioactive ingredients from the fruit extract.

Gas Chromatography – Mass Spectrometry

The Gas Chromatography – Mass Spectrometry (GC-MS) analysis was performed on a combined GC-MS instrument (ITQ 900 Model of Thermo Fisher Scientific make) using a HP-5 fused silica gel capillary column. The method to perform the analysis was designed for both GC and MS. 1 µL aliquot of sample was injected into the column using a PTV injector whose temperature was set at 275°C. The GC program was initiated by a column temperature set at 60°C for 5 min, increased to 300°C at a rate of 8 °C/min, held for 10 min. Helium was used as the carrier gas (1.5 mL/min). The mass spectrometer was operated in EI mode with mass source was set at 200°C. The chromatogram and spectrum of the peaks were visualized.

RESULT

Phytochemical Screening

In the present study preliminary phytochemical screening has done in the wild variety of *Coccinia indica* fruits. The phytochemical screening from the fruits of wild variety of *Coccinia indica* revealed that the presence of potential phytonutrients present in the domesticated *Coccinia indica* which has been reported in the previous. The extract was subjected to qualitative chemical analysis. Alcoholic extract answered positively for steroids, terpenoids, saponins, flavonoids alkaloids, tannins, glycosides, phenol and mucilage compounds which were shown in Table 1.

Gas Chromatography - Mass Spectrometry (GC-MS)

The results of GC-MS analysis of the alcoholic extract of the fruits of wild variety of *Coccinia indica* lead to the identification of a number of compounds. These compounds were identified through mass spectrometry attached with GC. The chromatogram and spectrum of the peaks were visualized (**Fig: 2**). The particular compounds present in the samples were identified by matching their mass spectral fragmentation patterns of the respective peaks in the chromatogram with those stored in the National Institute of Standards and Technology Mass Spectral database (NIST-MS, 1998) library.

The present study predicted the formula and structure and molecular weight of eight bio-molecules which has been shown highest peak compare to the other peaks. The name of the compounds and their molecular formula, molecular weight, peak area (%) and retention time were tabulated in **Table: 2**. The GC-MS graph and their molecular structure of these eight identified compounds were illustrated.

DISCUSSION

There is no literature exist in wild variety of *Coccinia indica* fruit so the present results were compared with reported results of domesticated *Coccinia indica* fruit and also to justify that wild variety *Coccinia indica* fruit can be substitute domesticated *Coccinia indica.* Since wild variety *Coccinia indica* fruits are bitter in nature therefore they can be utilized for the therapeutic properties which have been demonstrated by the researchers in domesticated *Coccinia indica.* The results of qualitative phytochemical profiling were clearly revealed that the plant contained different bioactive compounds such as Steroids, Terpenoids, Saponins, Flavonoids Alkaloids, Tannins, Glycosides, Phenol and Mucilage which are key compounds for the medical properties as per the existing literature. Earlier studies by various researchers also supported that these compounds were identified for the medicinal value of the plants. Also identified that these compounds play role in curing the diseases (diabetic, cancer, malarial, inflammation and

tuberculosis) through animal studies using different extracts of the plant.

The investigation by[7] demonstrated that Saponin and Flavonoid are found to be accountable for anti-diabetic activity of *Coccina indica* the present study of wild variety *Coccinia indica* fruit also confirmed the presence of Saponin and Flavonoid.

The study carried out by[8] demonstrates the significance of *Coccinia indica* as anti-inflammatory agent. 60% methanolic extract contained Flavonoid which can be responsible for the inhibition of prostaglandin synthesis which influenced anti-inflammatory activity in the tested rats. Therefore, the present study results represented that the presence of Flavoinds in the alcoholic extract of wild variety of *Coccinia indica* fruit also can be utilized as anti-inflammatory agent.

The previous study by[9] showed that wound healing potency of *C. indici*a be attributed to the phytoconstituents such as Tannins, Flavonoids, Terpenoids, and Saponins present in it, which may be either due to their individual or additive effect that fastens the process of wound healing. Correspondingly the results of phytochemical screening of alcoholic extraction of wild variety *Coccinia indica* fruit revealed the presence of these phytochemicals which prominently shows the wound healing property of wild variety *Coccinia indica.*

The work results showed that the fruit extract of *C. indica* mucilage has higher efficiency in removing high turbidity in comparison with low turbidity[10].The coagulation efficiency of *C. indica* mucilage in the study was found to be dependent on initial turbidity of water samples. The coagulation efficiency of *C. indica* mucilage in that study was found to be dependent on initial turbidity of water samples. The current study of wild variety *Coccinia indica* fruit also proved the presence of mucilage, so this extraction also can be used as sewage water treatment.

Phytochemical screening of *Coccinia grandis* fruit powder in organic solvent (methanol) revealed the presence of alkaloids (0.22±0.07mg), flavonoids (1.09±0.00mg), saponin (0.05±0.01mg) and phenol (17.01±0.02mg). Anti-inflammatory activity was assessed by Human red blood cell membrane stabilization assay (HRBS) by varying concentration of the fruit extract ranging from 100mg/ml to 500mg/ml. The results obtained in the study showed that the fruit extract of *Coccinia grandis* showed 44.46±0.09% and 84.63±0.04% anti-inflammatory activity for 100mg/ml and 500mg/ml concentration respectively, and the activity was found to be concentration dependent and proved folklore practice of the fruit extracts in curing various inflammatory responses[11].

The growing interest in recent years in medicinal plant research has been to a greater extent due to rapid progress in experimental techniques, as well as to a growing realization of the vast potential of plant based resources.

From the GC –MS results indicated that the presence of many bio-active compounds in wild variety *Coccinia indica* fruit. The GC- Mass Spectrometer investigated the compounds elute at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprint of that compound which can be identified from the data library.

With reference to Mass Finder Library of Terpenoids, it is confirmed that the following compounds are belongs to the group of Terpenoids. P-Methoxybenzoic acid, tetradecyl ester; Molecular Formula: $C_{22}H_{36}O_3$; Molecular Weight: 348, (6Z)-Nonen-1-ol; Molecular Formula: $C_9H_{18}O$;

Molecular Weight: 142, 2-octyn-1-ol; Molecular Formula: $C_8H_{14}O$; Molecular Weight: 126. 2,4-pentadien-1-ol, 3-pentyl-(2Z); Molecular Formula: $C_{10}H_{18}O$; Molecular Weight: 154 this is identified as Terpineol which is also belongs to the Terpinoid group.

Terpenoids are found to be responsible for anti-diabetic activity[7]. Plant terpenoids are widely used as industrially relevant chemicals, including many pharmaceuticals, flavours, fragrances, pesticides and disinfectants, and as large-volume feed stocks for chemical industries. Recently, there has been a renaissance of awareness of plant terpenoids as a valuable biological resource for societies that will have to become less reliant on petrochemicals[12]. GC-MS results of wild variety *C. indica* fruit indicated the presence of terpenoids from the bio-compound identified which confirms the various properties has been proven by the researchers.

The carried out the study on Terpineol has important anti-inflammatory and anti hypernociceptive properties. These effects seem to be associated with the power of Terpineol to inhibit the cytokine cascadegenerated by carrageenan and/or decrease the production of inflammatory mediators, as well as inhibit Nitric Oxide release[13]. Thus, a-terpineol can be an interesting candidate for the development of new drugs for treating painful conditions associated with inflammation. GC-MS results of wild variety *C. indica* fruit indicated the presence of terpineol from the bio-compound identified which validate the range of properties have been demonstrated in the previous works.

An approach used in the search for natural substances that possess therapeutic value is ethnobotany or ethnopharmacology. Active substances that have phenolic groups in their structure have great pharmacological potential. To establish a quantitative relationship between the species popularly considered to be anti-microbial, anti-diabetic, and anti-diarrheal, the contents of tannins and flavonoids were determined[14].

Despite the broad use of *C. indica* in traditional medicine, very few systematic pharmacological and phytochemical studies are reported till date assessing its therapeutic properties. The present study is the inauguration for the further details of pharmacological study on wild variety of *Coccinia indica.*

CONCLUSION

The current study reported the phyto-costituents of wild variety of *Coccinia indica* fruit for the first time. Alcoholic extract of wild variety of *Coccinia indica* fruit answered positively in qualitative phytochemical analysis. Further Gas Chromatography – Mass Spectrometry (GC-MS) was used for identification of bio-active compounds. The results of wild variety of *Coccinia indica* fruit were judged against previous literatures of *Coccinia indica* to substantiate that wild variety *Coccinia indica* has equivalent pharmacological properties. In view of the fact that wild variety of *Coccinia indica* fruit is not exposed or utilized for medicinal property hitherto. This study will be renaissance and ready to lend a hand for further detail analysis of wild variety of *Coccinia indica* as medicinal plant.

Conflict of Interests

The authors declare that there is no conflict of interests.

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Table 1. Qualitative Analysis of the Phytochemical from the Alcoholic Extraction of Wild Variety of *Coccinia indica* fruit.

PHYTOCHEMICALS	RESULT
STEROIDS	+
TERPENOIDS	+
SAPONINS	+
FLAVONOIDS	+
ALKALOIDS	+
CARBOHYDRATES	-
TANNINS	+
GLYCOSIDES	+
PHENOLS	+
MUCILAGE	+

+= Presence of Phytochemical; - = Absence of Phytochemical

Table 2. Compounds Identified from GC –MS of the Alcoholic Extract from Wild Variety of *Coccinia indica* Fruit.

Compound No.	Components	Molecular Formula	Molecular Weight	Peak Area (%)	Retention Time
1	2-amniophenyl,N-(tert-butyldimethylsilyl)	$C_{18}H_{25}NSi$	283	4.92	0.05
2	P-Methoxybenzoic acid, tetradecyl ester	$C_{22}H_{36}O_3$	348	6.18	0.07
3	(6Z)-Nonen-1-ol	$C_9H_{18}O$	142	7.20	0.10
4	Benzene, 2-benzyl-1-methyl-3-nitro	$C1_4H_{13}NO_2$	227	8.60	0.07
5	[1,1'-bicyclopropyl] 2-octanoic acid, 2'-hexyl-methyl ester	$C_2H_{38}O_2$	322	9.81	0.10
6	2-octyn-1-ol	$C_8H_{14}O$	126	10.88	0.06
7	2,4-pentadien-1-ol, 3-pentyl-(2Z)	$C_{10}H_{18}O$	154	12.55	0.06
8	3-Buten-2-one,4-(2,2,6,7- tetramethyl-7-azabicyolo[4.10] heptan-1-yl)	$C_{14}H_{23}NO$	221	14.06	0.08

* **Source**: *From the site of collection.*

Systematic Position of *Coccina indica*	
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliophyta
Order	Cucurbitales
Family	Cucurbitaceae
Genus	*Coccinia*
Species	*indica*

Fig: 1 *Coccinia indica*

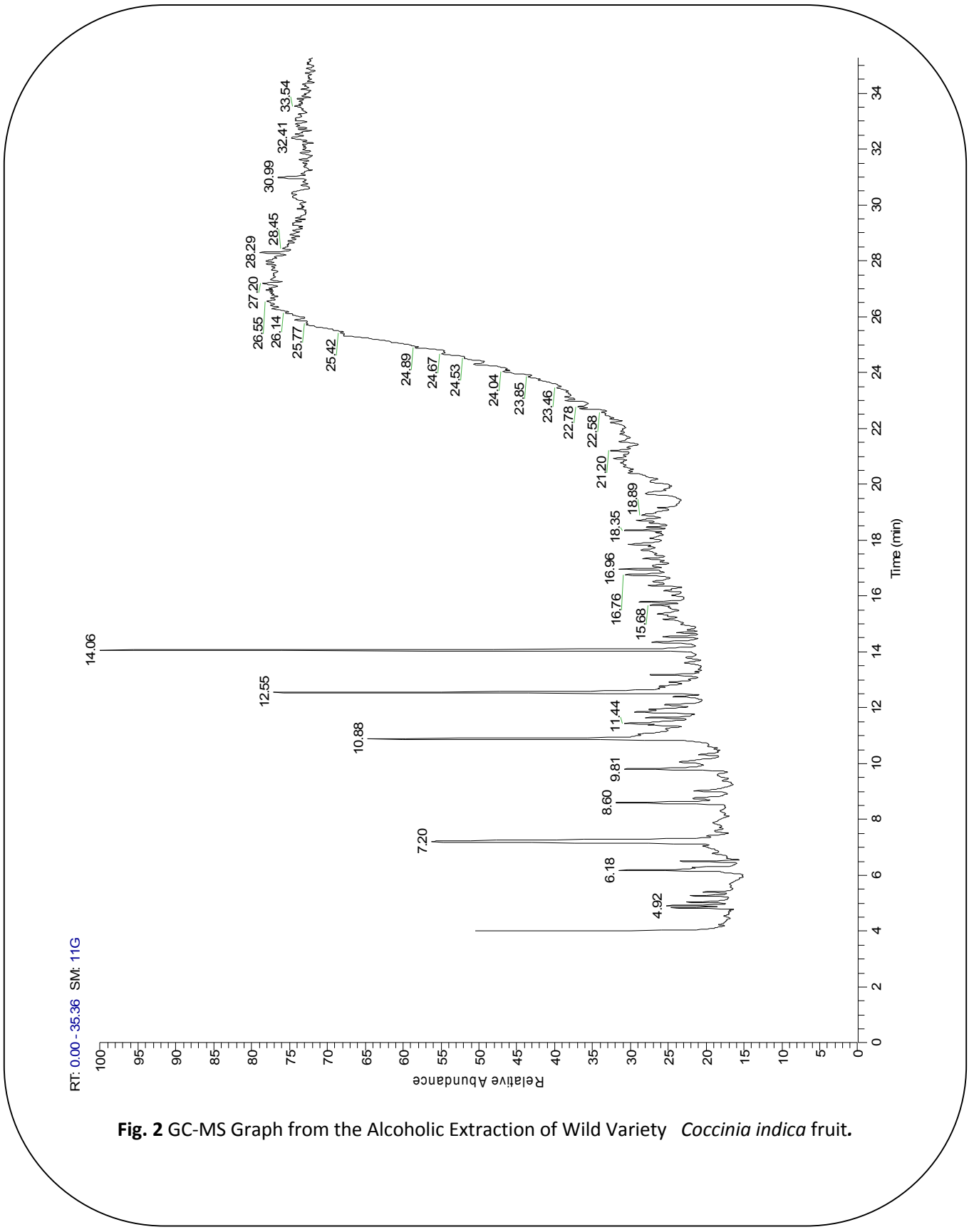

Fig. 2 GC-MS Graph from the Alcoholic Extraction of Wild Variety *Coccinia indica* fruit**.**