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RESEARCH ARTICLE

Phytochemical investigation of *Trichosanthes cucumerina* linn for Analgesic Activity

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ABSTRACT:
Trichosanthes cucumerina linn is used as a traditional medicine for various diseases. In the present study was conducted to evaluate theanalgesic activity for Petroleum ether and Methanol extract of *Trichosanthes cucumerina*linn and the activity was compared with diclofenac sodium as a standardand assessed using acetic acid induced abdominal writhing in mice.The methanolic extract exhibited significant value (**P<0.001**)analgesic activity as evidenced by increased the percentage of reduction in reaction time. The results thus support the*Trichosanthes cucumerina* linn used as ananalgesic agent. The plant showed no sign of toxicityup to the dose of 100 mg/kg in mice.

KEYWORDS: *Trichosanthes cucumerina* linn, Analgesic activity,Phytochemical screening, structural elucidation, IR.

INTRODUCTION:

The emerging new technologies have significantly contributed in the advancements in developing new phytopharmaceuticals and food herbs, which are definitely going to alter the future outlook of family physicians and common people. India can play major role in the global market for herbals, herbal products, raw materials and isolated phytopharmaceuticals because of its extensive flora and fauna, expertise, trained technocrats and great plant heritage from Ayurveda and other other resources. (1)

The curiosity of the present day man probes into the past and brings to light even fragmentary information about traditional methods of our ancestors, and it makes a fascinating study. The world health organization (WHO) estimates that 4 billion people 80 percent of the world population, presently use herbal care.

Herbal medicine is a major component in all indigenous peoples traditional medicine and a common element in ayurvedic, homeopathic, naturopathic, traditional oriental, and native American Indian medicine. WHO notes that of 119 plant-derived pharmaceutical medicines, about 74 percent are used in modern medicine in ways that correlated directly with their traditional uses as plant medicines by native cultures. Major pharmaceutical companies are currently conducting extensive research on plant materials gathered from the rain forests and other places for their potential medicinal value. (2)

In Asia there are many traditional systems, they are siddha and Ayurveda which are purely Indian systems. In india, the ayurvedha system of medicines was firmly believed to have originated from the Vedas and ancient religious scripts. In fact, there were strong convincing and asserting claims that ayurveda was a divine gift and celestial benediction to the Indian people. In short, the magic of herbs and plants are there all around us waiting to be discovered, understood and used. Because, they are now definitely recognized and accepted as perennial storehouses of infinite, limitless benefits to man.(3)

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Every herbal formulation must be standardized as per WHO guidelines. WHO collaborates and assists health ministries in establishing mechanisms for the introduction of traditional plant medicines into primary healthcare programmes, in assessing safety and efficacy and in ensuring adequate supplies and the quality control of raw and processed materials. HI According to WHO guidelines less stringent selection procedures could be applied for the screening, chemical analyses, clinical trials and regulatory measures but the procedure for pure phytochemicals for quality control should be identical to that for synthetic drugs according to WHO guidelines.

The world health organization (WHO) has recently defined traditional medicine as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today. The traditional preparations comprise medicinal plants, minerals, organic matter, etc. The number of patients seeking alternate and herbal therapy is growing exponentially. Herbal medicines are the synthesis of therapeutic experiences of generations of practicing physicians of indigenous system of medicine for over hundreds of years. Herbal medicines are now in great demand in the developing world for primary health care not because they are inexpensive but also for better cultural acceptability, better compatibility with the human body and minimal side effects. Thousands of year's traditional use can provide us with valuable subjected to selection, preparation and application of herbal formulation, to be accepted as viable alternative to modern medicine, the same vigorous method of scientific and clinical validation must be applied to prove the safety and effectiveness of a therapeutic product in the present study. We attempted to describe the present scenario and project the future of herbal medicine. (4,5)

***Trichosanthes cucumerina* (Snake gourd):**

The plant including roots, leaves, fruits, seeds have medicinal properties. The root is used as a cure for bronchitis, headache and boils. Both root and fruitare considered to be cathartic. The fruit is used as an anthelmintic. The seeds are used for stomach disorders and are also considered as antifebrile and anthelmintic. Studies on the pharmacological profile have shown the presence of anti-inflammatory activity in the roots and tubers and antidiabetic activity in seeds.(6,7,8)

MATERIALS AND METHODS:

Collection of plant materials:

The plants of *Trichosanthes Cucumerina linn* were collected from madurai during the months of December and identified by Dr.Stephen (Professor, American college, Madurai). The plants were then washed with water to remove soil and other extraneous matter. The leaves of plant were cut into small pieces and were dried under shade for 20 days. Then the dried material was homogenized to coarse powder and stored in airtight container.

Chemicals and solvents:

Petroleum ether AR, Methanol AR, Silica gel these are purchased from sisco laboratories, Chennai. Diclofenac sodium (standard), Acetic acid (1%v/v) these were purchased from sigma Aldrich, Bangalore.

Animals:

Adult healthy swiss albino mice of either sex weighing 25 - 30gm were used in the study. Each group contains 5 animals and the animals were housed in the animal house given a standard pellet diet and water. All animals were treated according to the standard procedures guided by NIH (7).

Analgesic activity of various extracts was evaluated by acetic acid induced writhing reflux in mice. Painful reaction in animals may be produced by the chemicals such as phenylquinone, bradykinin etc. Like that, acetic acid pain reaction which is characterized as a writhing response. Construction of abdomen of trunk (twist) and extension of hind legs are taken as reaction to chemically induced pain. Analgesic(both narcotic and non-narcotic) inhibit writhing response.

ANALGESIC ACTIVITY:

Method of extraction of chemicals:

About 400gms of dry coarse powder was soaked with petroleum ether (3000ml) for two days. After this, materials were extracted with petroleum ether (40^oC – 60^oC) by hot continuous percolation method for 72 hrs. The petroleum ether extracts were filtered and concentrated under reduced pressure. A green-black residue was obtained (40gms). The marc left after the petroleum ether extraction then dried and extracted with chloroform (2500ml) for 72hrs. The chloroform extract were also filtered and concentrated under reduced pressure. A dark black residue was obtained (50gms). Crude extracts were stored in desiccators. Then marc left after the chloroform extraction then dried and extracted with methanol (2500ml) for 72hrs. The methanol extract were also filtered and concentrated under reduced pressure. A dark green residue was obtained (35gms). Crude extracts were stored in desiccators. Analgesic activity of various extracts was evaluated by acetic acid induced writhing reflux in mice. Acetic acid pain reaction which is characterized as a writhing response.

Table No 1 -Data Showing The Preliminary Phytochemical Screening of ThePet.ether and Methanol Extract of *Trichosanthes Cucumerina Linn*

S. No.	Constituents	Pet.Ether Extract	Methanol Extract
1	Carbohydrate	-	+
2	Glycosides	+	+
3	Alkaloids	-	-
4	Flavanoids	+	+
5	Flavones	-	+
6	Steroids	+	-
7	Protiens and amino acids	-	+
8	Taniins	+	-
9	Saponins	-	+
10	Coumarins	-	+

+ → indicates positive test results- → indicates negative test results.

Method of analgesic activity:

The animals are grouped as follows and are treated according to the treatment protocol given below,

Tretment protocol:

Group-1 Treated as normal control received 10ml/kg of normal saline through orally.

Group-2 Treated as standard control received 10mg/kg of Diclofenac sodium through intraperitoneally.

Group-3 Treated as treatment control received 100mg /kg of Pet .ether extract of Trichosanthes cucumerina Suspended with 2ml of 1% CMC, administered through Orally.

Group-4 Treated as treatment control received 100mg/kg of Methanolic extract of Trichosanthes cucumerina Suspended with 2ml of 1%CMC, Administered through Orally.

All the extracts were administered half an hour prior to the acitic acid administration. Note the onset on writhing. Record the numbers of abdominal contractions, trunk twist and extension of hind limbs as well as the number of animals showing such response during a period of 10 minutes were noted.

STATISTICS:

Data are expressed as mean± SEM; data analyzed by one way ANOVA followed by Newman's Keul's multiple range tests to determine the significance between the control group and mice treated with the extracts.

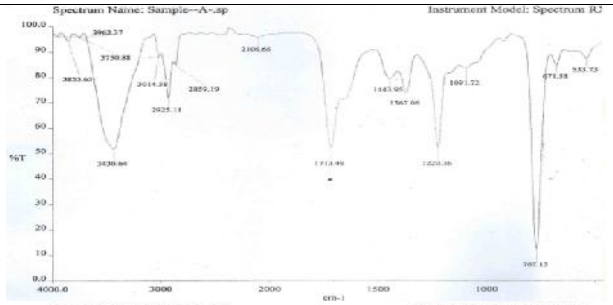
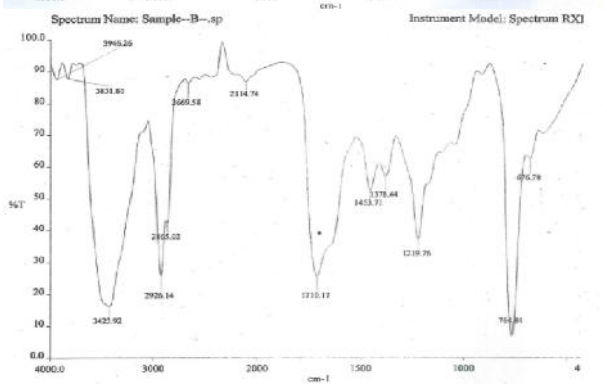
Table No.2 - Analgesic Activity of Various Extracts of *Trichosanthes Cucumerina Linn*By Acetic Acid Induced Writhing Reflux in Mice

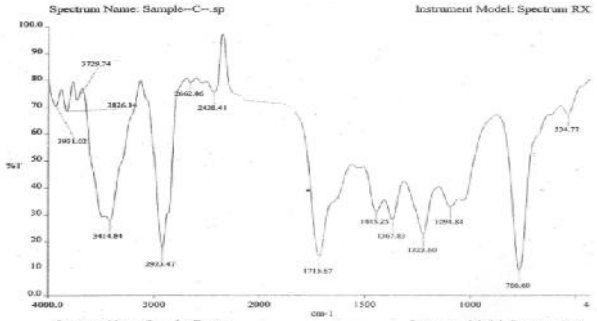
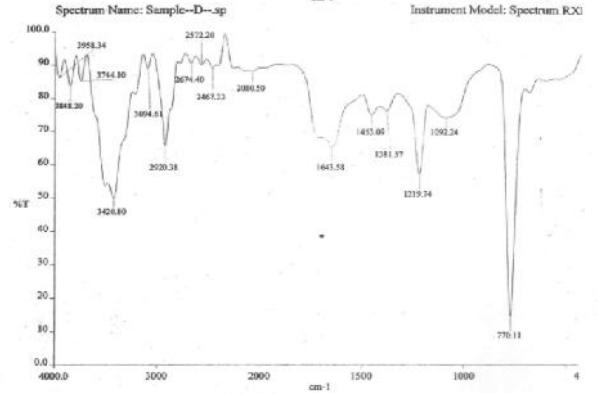
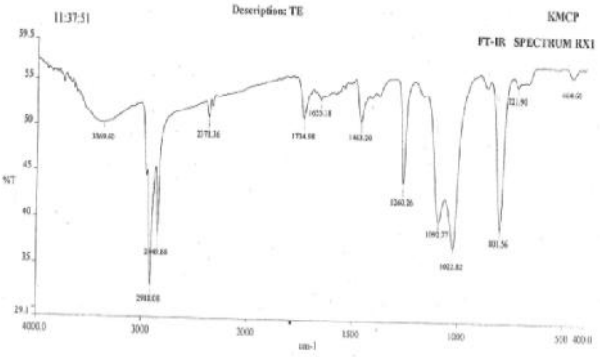
Treatment	Dose(mg/kg)	No of writhing	%reduction in reaction time
Group I - Normal saline	Inject1%v/v acetic acid1ml/100g of body weight	38±3.2	-
Group II - Standard	10mg/kg I.P. Diclofenac sodium	7±0.6	81.5%
Group III -Pet ether extract	100mg/kg Administered through orally	26±3.0	31.57%
Group IV -Methanolic Extract	100mg/kg Administered Through orally	10.6±1.6	73.68%**

Values are expressed as mean ± SEM

Values are find out by using one way ANOVA followed by Newman's Keul's multiple range test.

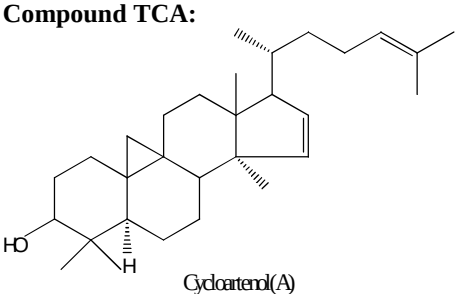
SPECTRAL ANALYSIS FOR ISOLATED COMPOUNDS

S.NO	COMPOUND	IR	GROUPS ASSIGNED
1.	**TCA**		Aromatic C-H Stretching May be due to O-H Stretching May be dueto C-H Stretching May be due to C=O Stretching May be due to Sp3 C-H Bending May be due to C-O Stretching May be due to C-N Vibration May be due to C-H Bending(opposite) May be due to N-H Bending(opposite)
2.	**TCB**		May be due to CH3 proton May be due to CH3 proton May be due to CH proton attached to alkyl group May be due to CH proton attached to alkyl group May be due to alylic proton (C=C) May be due to CH2 proton adjacent to C=O May be due to CH2 proton adjacent to C=O May be due to OH (Ester proton) May be due to Ester proton May be due to acyclic non conjucated bond May be due to CH2 proton attached to ethylinic bond

			May be due to aromatic nature
3.	**TCC**		Aromatic C-H Stretching May be due to O-H Stretching May be due to C-H Stretching May be due to C-H Bending May be due to C=O Stretching May be due to Sp3 C-H Bending May be due to C-O Stretching May be due to C-N Bending(opposite) May be due to C-H Bending(opposite)
4.	**TCD**		Aromatic C-H Stretching Aromatic C-H Stretching Aromatic C-H Stretching May be due to O-H Stretching May be due to Sp3 C-H Stretching May be due to C-H Stretching May be due to C-H Bending May be due to C=C Stretching May be due to Sp3 C-H Bending May be due to C-O Stretching (aryl, alkyl, ether) May be due to C-N Bending(opposite) May be due to C-H Bending(opposite)
5.	**TCE**		May be due to O-H Stretching May be due to Sp3 C-H Stretching May be due to Sp3 C-H Stretching May be due to C=O Stretching May be due to Sp3 C-H Bending May be due to C-O Stretching (aryl, alkyl, ether) May be due to C-N Bending(opposite) May be due to C-N Bending(opposite) May be due to C-H Bending(opposite Aromatic

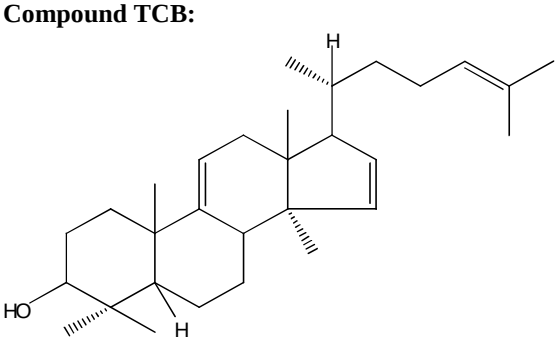
The isolated compounds are as follows compound A, B, C, D and E:

Compound TCA:



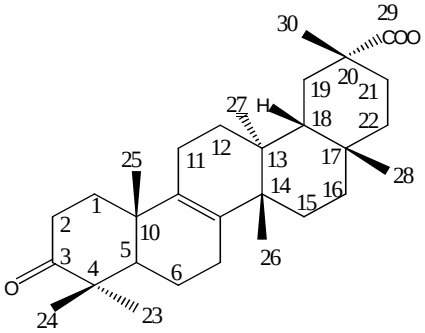
Cycloartenol(A)

Compound TCB:

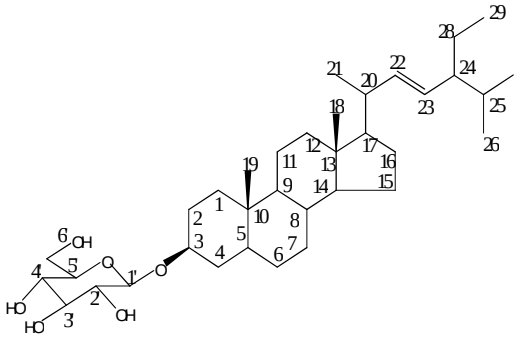


Parkeol (H)

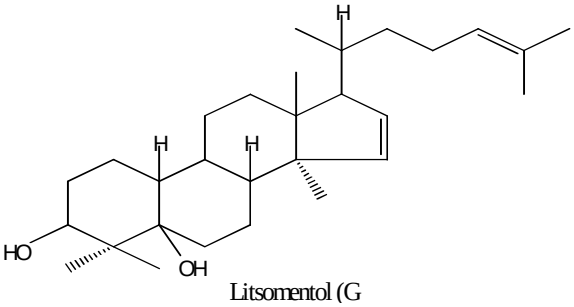
Compound TCC:



Compound TCD



Compound TCE



Litsomentol (G

RESULTS AND DISCUSSION:

The table 2 value shows that the analgesic activity of various extracts of *Trichosanthes cucumerina* linn by acetic acid induced writhing reflex. The result reveals that Pet. ether extract does not possess analgesic activity when compared with methanolic extract of *Trichosanthes cucumerina* linn. But among this two extracts methanolic extracts possess more significant analgesic activity at P<0.001 than pet ether extracts[9-13]. The methanolic extract of *Trichosanthes cucumerina linn* is very effective as the standard analgesic diclofenac.

Our study has proved that the analgesic activity of the ***Trichosanthes cucumerina*** in concordance with the previous study[14]. This study shows that the methanol extract shows good analgesic activity in concurrence with the previous studies[15]. When comparing with standard methanolic extract gives effective pharmacological action.

CONCLUSION:

Medicinal plants have been identifided and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological compounds and used to perform important biological functions[16,17]. Therefore this study concludes that the methanolic extract of *Trichosanthes cucumerina* linn has the potency to treat for analgesic effect in the animal study. In later stage this methanolic extract of *Trichosanthes cucumerina* linn. may also be used as an analgesic. The presence of flavonoid, saponins and coumarins was identified might be responsible for the analgesic activity in methanolic extract.

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