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PHARMACOLOGICAL SCREENING OF ANTI HELMINTIC ACTIVITY, ANTI BACTERIAL ACTIVITY AND ANTI OXIDANT OF CANTHIUM COROMANDELICUM

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Abstract:

Day by day helminthiasis and bacterial infections were increasing globally. The major aim of the study is to investigate preliminary phytochemical screening, anti-bacterial, antioxidant and anti-helminthic activity of different extracts of Canthium coromandaelicum. Preliminary phytochemicals analysis of the extract showed the presence of alkaloids, flavonoids, saponins, and taninns. The evaluation of the anti-helminthic activity is done by using the adult Indian earthworms (Pheritima posthumas it has similar anatomical and physical resemblance of intestinal round worm. The different concentrations of different extracts (Ethanolic Extract, Methanolic Extract and Aqueous alcoholic extract of Canthium coromandelicum) is compared to standard Piperazine Citrate. And then the time for paralysis and time taken for death of the worms were noted. The Present Study is designed to evaluate the Protective effects of different extracts of Canthium Coromendalicum against various activities like Anti-oxidant activities, Anti-bacterial and antihelminthic activity. Evaluation of the anti-bacterial activity was done by cup plate method at different concentrations (25mg/ml, 50mg/ml, 75mg/ml and 100mg/ml) of extract and the results were compared with gentamycin (standard antibiotic). After 48hrs we observed the zone of inhibition. By zone of inhibition, it was proved that it is having the good anti-bacterial activity. As well as the Antioxidant Activity was proved by DPPH Assay. DPPH scavenging activity of plant extract (IC50 13µg/ml) showed IC50 value close to the ascorbicacid(15µg/ml). Ethanolic extract has showed better effects than Aqueous alcholic extract and methanolic extract in Antioxidant Study..

KEYWORDS: *Canthium coromandelicum, anti-bacterial activity, anti-helminthic activity, preliminary phytochemical screening, anti-oxidant activity.*

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INTRODUCTION:

Helminthiasis, also known as worm infection, is any macroparasitic disease of humans and other animals in which a part of the body is infected with parasitic worms, known as heliminthis. Soil-transmitted helminthiasis and schistosomiasis are the most important heliminthiasis [1].

Soil-transmitted heliminth infections can causethe significant burden.It is estimated that 1.5 billion people worldwide are infected with these infections[2]. Prevalence of the soil-transmitted heliminth infections in India about 27% with substantial heterogeneity [3].

The prevalence rate of the heliminthiasis is vigorously increasing[4]. The use of herbal medicines has been increased because the synthetic substances having the toxic effects[5] Canthium coromandelicum, also known as karai, it belongs to the family Rubiacea, it is a bushy thorny suffruticose herb,a native of India found mainly in the coramandel region[6]. It having the various medicinal uses like in treating wounds, scabies, fever, diuretic problems, diabetes, indigestion, diarrhea, snakebite and bloodpurification.It also having the anti-oxidants. It having the chemical composition of palmitic acid, n-pentacosane, squalene, octacosane, heptacosane, n-tetracoasane, hexacosane, phytol, sitosterol, nonacosane[7].

The phytochemicals like tannins was outlined as having antihelminthic activity. It having the capability to bind with the free proteins in the gut of the host or glycoproteins on the cuticle of the parasite and this leads to death of the parasite[8].

MATERIALS AND METHODS:

PREPARATION OF PLANT MATERIAL

The leaves of the Canthium coromandelicum(Rubiaceae), was collected from the local areas of Rajamahendravaram, Kadiam, Chagallu, Thungapadu, of Andhra Pradesh in the month of the January 2025. The plant material was authenticated by the Dr. D. Narendra, Principal, Department of Pharmacognosy, VJ's College of Pharmacy.

PREAPARTION OF EXTRACT

The leaves of the plant was dried in shade and made into a fine powder by using motor and pestle. The dry powder is extracted with different solvents like ethanol, Methanol and aqueous alcoholic extract using maceration process for 48hrs.

PHYTOCHEMICAL TESTS

The preliminary phytochemical tests revealed the ethanolic extract, methanolic and aqueous alcoholic extract of the leaves showed the presence of the phytoconstituents as tabulated in table [9].

EXPERIMENTAL ANIMALS

Adult earthworms (Pheritima posthuma) were used to evaluate anti helmintic activity in vitro. Earthworms were collected from the VJ's College of Pharmacy and were washed with normal saline to remove all the faecal matters were used for the anthelmintic study. The earthworms of 3.5cm in length and 0.1-0.2 cm in width were used for all the experimental protocol. All the test solution and standard drug solution were prepared freshly before starting the experiments. Observations were made for the time taken for the paralysis was noted when no movement of any sort could be observed except when the worms were collected and kept in normal saline solution [10].

EVALUATION OF ANTI HELMINTIC ACTIVITY:

CHEMICALS AND REFERENCE DRUGS

For the antihelmintic test, the different extracts of Canthium coromandelicum was tested using different concentrations. Piperazine Citrate was used as the standard drug for evaluation of anti helmintic activity.

COLLECTION OF THE WORMS

Healthy adult Indian earthworm (pheritima posthuma) about 3-5cm in length and 0.1-0.2cm in width and weighing about 0.8-3.04g.

REFERENCE STANDARD: Piperazine citrate20mg/ml

EXPERIMENTAL MODEL

For the investigation, Indian earthworm pheritima posthuma, were used to study the anti helimintic activity were collected from the water logged areas of soil at VJ's College of Pharmacy. about 3-5cm in the length and 0.1 - 0.2cm in width and weighing about 0.8- 3.05g. The earthworms resembled the intestinal roundworms parasites of human beings both anatomically and physiologically and hence were

used to study the antihelmintic activity.

ANTIHELIMINTIC ASSAY: The test was carried out by using the adult earthworm (pheritimaposthuma) because of its anatomical and physiologiacal similarly with the intestinal round worm parasite of human beings. All the worms were washed with normal saline water to remove all the fecal matters present on their bodies. Extract was weighed and dissolved in 10ml of DMSO to obtain the concentrations of 100mg/ml, 200mg/ml, 500mg/ml and 1000mg/ml. Earthworms were divided into five groups(each group containing five worms) in a petri dish. The different extracts of Canthium coromandelicum was added to the petri dishes and the time of paralysis was determined. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms was recorded after ascertaining those worms neither moved when shaken vigorously nor when dipped in arm water (50ºc) followed with their fading away of their body colors.

EVALUATION OF ANTI-BACTERIAL ACTIVITY

CHEMICALS AND REAGENTS USED; Gentamycin, DMSO (dimethyl sulfoxide), sodium chloride, peptone, beef extract, and dextrose.

TEST BACTERIAL STRAIN USED:

Gram positive bacteria: Staphylococcusaurens

REFERENCE STANDARD: Gentamycin 80mg/2ml

PR**EPARATION OF NUTRIENT AGAR MEDIA:**

Beef extract (0.6gm) sodium chloride(0.10gm) peptone(0.10gm), agar(3gm) and distilled water (200ml) are placed in a conical flask and added 200ml of distilled water. The ingredients are dissolved by heating on a water bath with stirring until the clear solution was obtained. PH was adjusted upto 7.6 and then filled by putting cotton plug into funnel immediately. When the solution is hot, distributed around 10ml into each test tube highly close the test tube with non-absorbent cotton and sterilized it by allowed autoclaving at 15lbs pressure 120^0c for 30minutes. After removing from the autoclave, allowed it to cool but not solidify. The nutrient agar was poured into petri plate and leave the plates on the sterile surface until the agar has solidified.

PREPARATION OF TEST AND STANDARD:

The stock solution of test compound was prepared by dissolving the ethanolic extract, methanolic and aqueous alcoholic extract of Canthium coromandelicum in DMSO.The stock solution of reference standard gentamycin was prepared at a concentration of 25mg/ml in sterile water.

Sterilization was carried out in autoclave at 15lbs for 20minutes. Agar media, water, etc.., were sterilized in autoclave[11].

ANTI BACTERIAL ASSAY BY CUP PLATE METHOD

30ml of sterile nutrient agar medium was poured into sterile petri dishes by spread plate techniqueand allowed to solidify. The petri plates were incubated at 37^0C for24hrs to check for sterility. The medium is seeded with the organism by pour plate method using sterile top agar(4ml) contained1ml culture.Borers were made on the medium using sterileborer. Dried extracts of Canthium coromandelicum was dissolved in DMSO to obtain different concentrations i.e., 100mg/ml, 300mg/ml and 500mg/ml. From 25mg/ml was taken as reference. All the plates were kept in a refrigerator at 2^0C to 8^0C for period of 2hours for effective diffusion of test compounds and standard. Later they were incubated at 37^0C for 24hrs. Zone of inhibition around the cups indicated anti bacterial activity12].

ANTIOXIDANTACTIVITY:

FREE RADICAL SCAVENGING ACTIVITY (DPPH):

DPPH is one of the free radicals widely used for testing preliminary radical scavenging activity of the plant extract. Scavenging of DPPH radical is related to the inhibition of liqid peroxidation. DPPH is usually used as a substance to evaluate the antioxidant activity. Antioxidants either transfer an electron or a hydrogen atom to DPPH thus neutralizing its free radical character. DPPH test, which is based on the ability of DPPH a stable free radical, to decolorize in the presence of antioxidants, is a direct and reliable method for determining radical scavenging action. The DPPH assay has been largely used as a quick, reliable and reproducible parameter to search the in vitro general antioxidant activity of pure compounds as well as plant extracts.

DPPH solution was prepared by dissolving 4mg in 100ml of methanol. Different concentrations of ascorbic acid and extract were prepared. Prepared solutions were mixed in DPPH and stored in dark place for 30min. Absorbance of the mixture was seen at 517 nm, absorbance is repeated for three times.The graph was extrapolated to find the 50%inhibition concentration of test sample and ascorbic acid, percentage inhibition was calculated [13].

%Inhibition=Absorbance of control–absorbance of sample/absorbance of control×100

STATISTICAL ANALYSIS

All the results were expressed as Mean ± S.E.M. (n=3) using GraphPad® prism (version 8.1.2(332)) software. Statistical analysis was performed using two way ANOVA and one way ANOVA followed by Tukey's multiple comparison test. $P < 0.002$ was considered as statistically significant.

RESULTS:

TABLE I: PHYTO CHEMICAL SCREENING

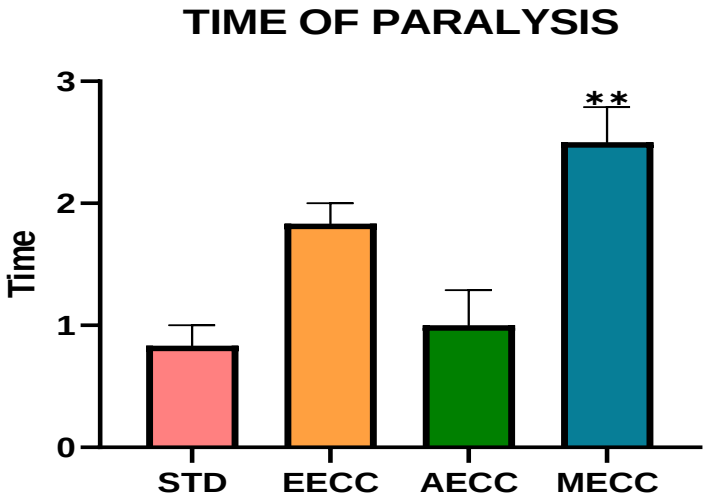
Phytochemical Screening of EECC, MECC & AACC showed the presence of Alkaloids, , Flavanoids, Tannins, Glycosides, Saponins and phenolic compounds.

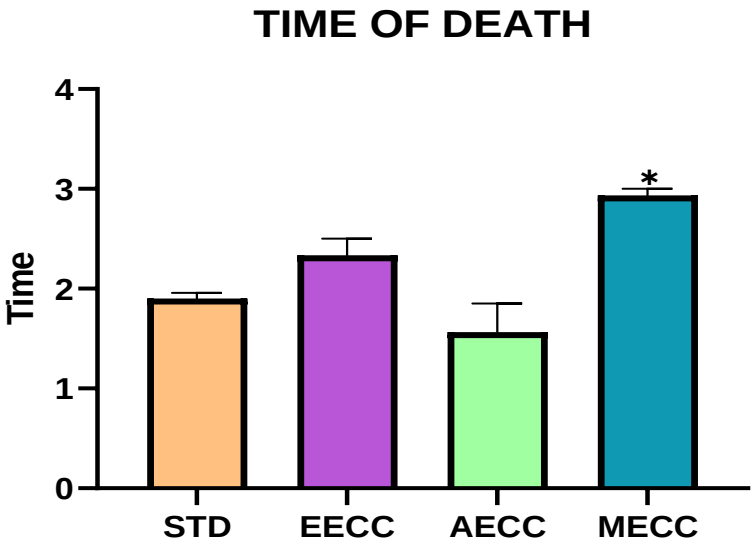
S.NO	PHYTOCONSTITUENTS	EECC	AECC	MECC
1	ALKALOIDS	+	+	+
2	GLYCOSIDES	+	+	+
3	PHENOLIC COMPOUNDS	+	+	+
4	TANNINS	+	+	+
5	SAPONINS	+	+	+
6	FLAVANOIDS	+	+	+

TABLE II: ANTI-HELMINTHIC ACTIVITY:

GROUPS	TIME OF PARALYSIS (in Min)	TIME OF DEATH(in Min)
STD	0.83±0.16	1.9±0.05
EECC	1.83±0.16	2.33±0.16
AECC	1±0.28	1.56±0.28
MECC	2.5±0.28	2.66±0.16

Results were expressed in Mean±SEM



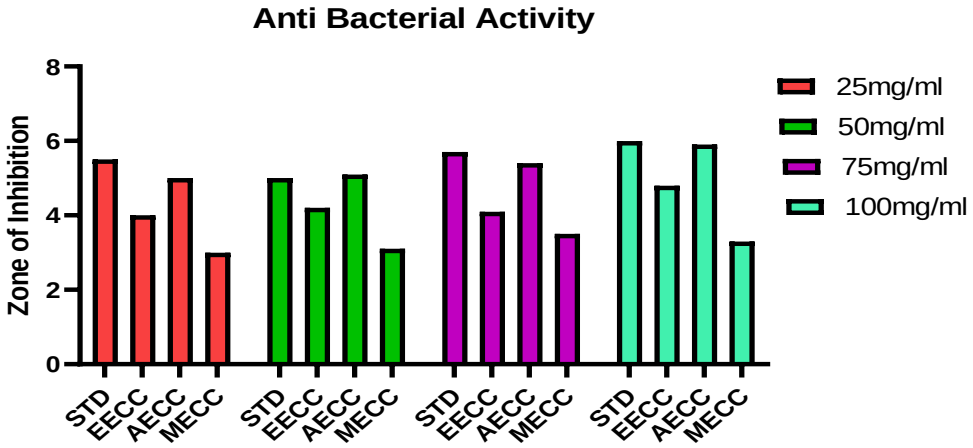


Values were expressed as Mean ± S.E.M., n=6 in each group. Statistical analysis was carried out by two way ANOVA followed by Tukey's multiple comparison test. Significant difference $^{**}p < 0.05$, $^{*}p < 0.05$ when compared to Standard Group.

INFERENCE: AECC Has showed the shortest time of paralysis (1.86±0.6) and the death (1.56±0.28) at the concentration of 100mg/ml. AECC shows the better action when it is compared with the EECC and MECC extract.

TABLE III: ANTIBACTERIAL ACTIVITY:

GROUPS	25mg/ml	50mg/ml	75mg/ml	100mg/ml
STD	5.5	5	5.7	6
EECC	4	4.2	4.1	4.8
AECC	5	5.1	5.4	5.9
MECC	3	3.1	3.5	3.3



INFERENCE: The AECC shows the zone of inhibition 5.9cm at the100mg/ml, it shows the nearest value to the standard value amongst other extract.

TABLE IV: ANTIOXIDANT ACTIVITY

GROUPS	ABSORBANCE	IC_{50}
STD	1.9	12µg/ml
EECC	1.95	13.7µg/ml
AECC	1.34	40.7µg/ml
MECC	1.654	26.8µg/ml

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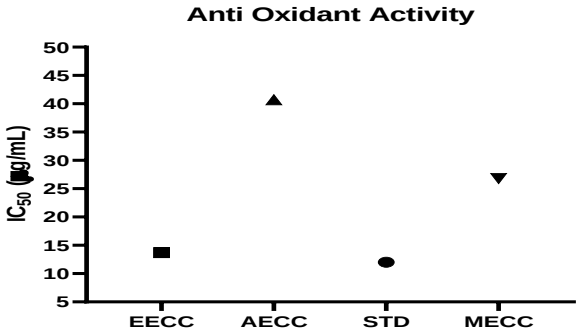

INFERENCE AECC showed 40.7µg/mL and MECC showed 26.8µg/mL where EECC showed IC50 13.7µg/mL, which is comparable to Ascorbic acid IC50 12 µg/mL

DISCUSSION:

All the tests in the current study were done in vitro. One of the primary benefits of analyzing the biological properties of plant extracts invitro is that the process is inexpensive and fast, allowing for large scale screening. The phytochemical screening of the different plant extract Canthium coromandelicum leaves shows the presence of alkaloids, glycosides, tannins, saponins, phenolic compounds, and flavonoids.

In the present study the phytochemical found such as alkaloids, saponins, tannins, phenols and other constituents have already proven for anti-helminthic activity. Tannins are previously reported that it may interfere with the worms energy production by the action of uncoupling oxidative phosphorylation or it bind to the free protein of the worms GI tract and it leads causing the death. The main action of the piperazine citrate is bind with the GABA receptors, the causes the hyperpolarization of the nerve endings, the leads to the flaccid paralysis of the worm. It expected that the phytochemical present in the Canthium coromandelicum extracts was show the similar effects, killing the worms. In the evaluation of the activity of the 3 extracts of Canthium coromandelicum. It has shown that all the three extracts has showed that the dose dependent activity with improved anti helminthic activity. In that we observe that the AECC shows the shortest time of paralysis (1.86±0.6) and the death (1.56±0.28) at the concentration of 100mg/ml. AECC shows the better action when it is compared with the AECC and MECC extract. This action shows may be due to the presence of phytoconstituents in different extracts. The phytochemical screening proved that AECC contains the alkaloids, saponins, glycosides, and other phytochemicals having the various activities. The anti-bacterial activity is done by the agar cup plate method and the results are measured by the zone of inhibition. The zone of inhibition of the standard and the plant extracts are compared and in this the standard drug (Gentamycin) shows the zone ofinhibitionabout6cmat the concentration 100mg/ml and the AECC shows the zone of inhibition 5.9cm at the100mg/ml,it shows the nearest value to the standard value amongst other extract. The oxidative stress is measured by DPPH scavenging assay. DPPH scavenging activity of plant extract (IC50 13µg/ml) showed IC50 value close to the ascorbic acid (15µg/ml).

CONCLUSION:

The aqueous extract of the Canthium coromandelicum leaves exhibited a better anti helminthic activity when compared with other extracts with shortest time of paralysis and death at the concentration of 100mg/ml. The AECC showed the presence of phytoconstituents like alkaloids, amino acids, tannins, carbohydrates, glycosides, saponins and steroids.The extract can be potent novel therapeutic strategy for anti-helminthicactivity.

The ethanolic extract of the Canthium coromandelicum leaves exhibited the better anti-oxidant activity when compared with the other extracts. The DPPH activity with the standard inhibitory concentration of 15µg/ml the EECC shows the nearest concentration 13µg/ml, so it shows the better anti-oxidant activity.

The anti-bacterial activity of the aqueous extract of Canthium coromandelicum leaves exhibited the better anti-bacterial activity compared with the other extracts. In this activity the zone of inhibition of the standard drug shows the 6cm of zone of at the 100mg/ml,the AECC shows the nearest value of zone of inhibition 5.9 at100mg/ml.So,theAECC has showed the better anti-bacterial activity when compared to other Groups.

CONFLICT OF INTERESTS

Declare none

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