EVALUATION OF ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACT OF *AGAVE CANTALA* ROXB

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ABSTRACT

In the present study methanolic extract of *Agave cantala* Roxb. (MEAC) was examined for anti-inflammatory and antioxidant properties using *in vivo* and *in vitro* methods, respectively. Anti-inflammatory activity was evaluated by Carrageenan and Dextran induced models (acute) and Granuloma cotton pellet (chronic) model. Anti-nociceptive activity was evaluated by acetic acid-induced writhing and hot plate method and Antioxidant was assessed by hydroxyl radical scavenging and FRAP assay. In acute model of inflammation as produced by Carrageenan (46.15% and 61.53%), by Dextran (20.56% and 26.33%) at doses of 200 mg/kg and 400 mg/kg protection from inflammation was observed. While in chronic anti-inflammatory model using cotton pellet granuloma (25.63% and 44.97%) at doses of 200 mg/kg and 400 mg/kg, protection from inflammation was observed. MEAC significantly reduced the number of writhing events induced by acetic acid solution at inhibitory rates of 32% and54% and hot-plate test indicate that MEAC (at doses of 200 and 400 mg/kg) significantly increased the latency of the response. The extract displayed potential inhibitory effect of hydroxyl radical-scavenging activity. The results of the ferric reducing assay indicated that MEAC had stronger reducing power. These results conclude that methanolic extract of *Agave cantala* Roxb reported anti-inflammatory and antioxidant activities, there by justifying its usage in folk medicine.

Keywords: Methanolic extract of *Agave cantala* (MEAC), Carrageenan, Cotton pellet, Dextran, Catechol.

1. INTRODUCTION

Agaves have great economic and cultural importance for several native and cross-breed communities in Mexico. *Agave cantala* is a perennial, stout, scapigerous herb [1]. *A. cantala* almost certainly originated in Mexico, where it is now rare. *A.cantala* was introduced by European traders into southern Asia, specifically India, where it was planted initially as a hedge and fence plant and to control erosion in some areas. It's leaves are a source of fiber, which is known as 'cantala', 'kantala' or 'cantula' fiber. The shoot buds, cut into pieces, are eaten as a cooked vegetable in Java. Various parts of the plant were reported to be used as laxative, emmenagogue, scurvy, syphilis, scrutula, swelling, retention of urine, anticancer, cytotoxic, diuretic, aphrodisiac, antisyphilitic in folklore claims [2].

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Inflammation is an immune response against pathogens, tissue injury and surgical trauma. This response gives rise to the initial cardinal signs of inflammation, which include redness, heat, swelling, pain and loss of function [3]. The study of inflammation is important because of its intimate link to other diseases such as arteriosclerosis, obesity, cancer, chronic obstructive lung disease and asthma [4].

The use of non-steroidal anti-inflammatory drugs (NSAIDS) is the most common treatment for inflammation. These drugs inhibit cyclooxygenase enzymes (COX-1 and COX-2) and consequently reduce prostaglandin levels [5]. However, these compounds provoke undesirable side effects such as peptic ulcer formation, bleeding and perforation of the gastric mucosa [6]. Glucocorticoids, which are alternative drugs for the treatment of chronic inflammation, can induce systemic adverse effects in chronic patients, such as adrenal insufficiency [7]. In this context, new drugs to treat inflammation with fewer side effects are necessary. Several medicinal plants have shown

promising results for alleviating pain. Many patients who use such treatments perceive them to be effective [8]. Different Agave species play an important role on inflammation process [9], for example *Agave intermixta trel* is used in the treatment of inflammatory arthritis and tumors [10], in other studies *Peana e colaboradores* (1997) [11] showed the anti-inflammatory potential of *A. americana.*

Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such asthiols, ascorbic acid, or polyphenols [12].

2. MATERIALS AND METHODS
2.1. Plant material
The whole plant of *Agave cantala* (Roxb.) was collected from surroundings of Tirupati, India in the month of December 2012 and authenticated by Dr. K. Madhava chetty, Assistant professor, S.V.University, Tirupathi. The voucher specimen is preserved in Department of Pharmacognosy, Sree Vidyanikethan College of Pharmacy for further reference.

2.1.1. Preparation of the extracts
The plant was collected, washed and dried at room temperature. After complete drying, it's leaves were powdered. Dried powdered drug was extracted with Methanol, by using soxhlet apparatus ($50-60^0C$).

2.1.2. Preliminary phytochemical screening of the extracts
The preliminary phytochemical tests were carried out for the extracts using standard phytochemical methods [13]**.**

2.1.3. Acute oral toxicity study
Adult Wister rats weighing 150- 200g were used for the study. The starting dose level of MEOA was 2000mg/kg body weight p.o. Dose volume was administered to overnight fasted rats with *ad libitum*. Food was withheld for further 3- 4 hours after administration and the animal were observed for behavioral changes, the animals were observed for a further 14 days for any signs for delayed toxicity. The onset of toxicity and signs of toxicity were not observed. On further study, no toxicity or death was observed at these levels.

Selection of dose:
The LD_{50} cut off value found to be 2000mg/kg. For the assessment of anti-inflammatory activity two dose levels were selected i.e., first dose is one-tenth of LD_{50} cut off value and second dose was twice that off one-tenth dose (200mg/kg & 400mg/kg p.o single dose)

2.2 EVALUATION OF ANTI - INFLAMMATORY ACTIVITY
Animals
Experimental animals (Wistar rats) weighing 150-170 g were selected. The animals were housed in standard polypropylene cages at room temperature of 20^0C-200C, under a 12-12 hr dark and light cycle. After one week of acclimatization, the experimental animals were divided randomly in to 4 groups (n=6). The experimental protocol was approved by the Institutional Animal Ethical Committee of Sree Vidyanikethan college of Pharmacy [(IAEC/I-002) of CPCSEA Reg. no.930/PO/a/2006/CPCSEA], Rangampet, Tirupati. The animals were divided into four groups (n = 6). Group I served as control receiving inflammatory inducing agent only. Group II served as Standard, Group III and IV served as test, receiving methanolic extract at doses of 200 and 400 mg/kg p.o. respectively.

1. Carrageenan induced rat paw oedema method
Acute inflammation was induced by sub plantar injection of 0.1 ml of Carrageenan (1% Carrageenan suspended in 0.9% Nacl) in the right hind paw of the rats. After one hour of administration of standard Ibuprofen (10 mg/kg) and methanolic extract of *Agave cantala* (dose of 200 mg/kg and 400 mg/kg), an injection of 0.1ml of carrageenan was given. Measurement of paw size was done by means of volume displacement technique using plethysmograph immediately before carrageenan injection and 30, 60, 120 and 180

min after the carrageenan injection and the percentage of anti-inflammatory activity was calculated. % inhibition of paw edema is compared with control group [14].

2. Dextran-induced rat paw oedema method

The treatment for animals and measurement of paw volume (30, 60 & 120 min) was done as described above, Dextran (0.1ml, in 0.9% NaCl) was used in the place of carrageenan [15] .Methanolic extract (200 & 400 mg/kg), Ibuprofen (10 mg/kg) were administered orally to study groups of rats, one hour prior to the dextran injection.

3. Cotton pellet induced granuloma method

This method was adopted by D'Arcy (1960) which was carried out by using sterilized cotton pellet implantation method in rats. After shaving off fur, the animals were anaesthetized. Sterile pre-weighed cotton pellets (20 ± 1 mg) were implanted in the axilla region of each rat through a single needle incision. Plant extract (200 & 400 mg/kg body wt.), Dexamethasone (0.5 mg/kg body wt.) and vehicle alone was administrated orally for seven consecutive days for the 4 groups, from the day of cotton pellet implantation. On the 8th day the animals were sacrificed by cervical dislocation, the cotton pellets were removed and made free from extraneous tissues. The pellets were dried at 60^0 C for 24 hrs. The percentage inhibition of the dry weight of the granuloma were calculated and compared.

2.3 EVALUATION OF ANTI-NOCICEPTIVE ACTIVITY

1. Acetic acid-induced writhing

Albino rats (n=6) were used according to a method described previously [16]. The total number of writhing events following intraperitoneal administration of 0.6% acetic acid was recorded for 15 min, starting 5 min after injection. The animals were pre-treated with MEAC at doses of 200 and 400 mg/kg (p.o.) and the controls were pre-treated with vehicle and Ibuprofen (10 mg/kg p.o.) 1 h before acetic acid administration. Antinociceptive activity was expressed as a reduction of the number of abdominal writhes relative to the negative control.

2. Hot plate test

The hot-plate test was performed to measure response latencies according to the method that was described by Eddy and Leimback (1953). The rats were first treated with different doses of A.cantala (200 and 400 mg/kg p.o) after 1 h of extract administration they were placed on a hot plate maintained at 55±1.0°C. A cut-off period of 15 s was considered as maximal latency to avoid injury to the paws. The time taken by the animals to lick the fore or hind paw or jump out of the place was taken as the reaction time. Ibuprofen (10 mg/kg) was used as a reference drug.

2.4 EVALUATION OF ANTIOXIDANT ACTIVITY

Determination of total phenols

The total phenolic contents methanolic extract of *Agave cantala* were determined according to the method described by Malik and Singh [17]. Aliquots of the extracts were taken in a 10 ml glass tube and made up to a volume of 3 ml with distilled water. Then 0.5 ml folin ciocalteau reagent (1:1 with water) and 2 ml Na_2CO_3 (20%) were added sequentially in each tube. A blue color was developed in each tube because the phenols undergo a complex redox reaction with phosphomolibdic acid in folin ciocalteau reagent in alkaline medium which resulted in a blue colored complex, molybdenum blue. The test solutions were warmed for 1 minute, cooled and absorbance was measured at 650 nm against the reagent used as a blank. A standard calibration plot was generated at 650 nm using known concentrations of catechol. The concentrations of phenols in the test samples were calculated from the calibration plot and expressed as mg catechol equivalent of phenol/g of sample.

Determination of total flavonoid content Standard curve of Quercetin

1 mg of quercetin was weighed and dissolved in 100 ml of distilled water and successive dilutions were made to make up the concentrations 2,4,6,8 and 10 mcg/ml. A volume from above aliquots was taken and mixed with 1.25 ml of FC reagent. It was left for 5mins. Then 2.5 ml of sodim carbonate was added and it was let to react for 30 min then the volume was made upto 10 ml. Then the absorbance was measured at 765 nm. The calibration curve was drawn plotting the absobance and concentrations.

The total flavonoid content was determined using the Down method. 5 ml of 2%

Aluminium trichloride ($AlCl_3$) in methanol was mixed with the same volume of the extract solution (0.4mg/ml). Absorption readings at 415 nm using UV-VIS spectrophotometer and readings were taken after 10 min against blank sample consisting of a 5 ml extract solution with 5 ml methanol without $AlCl_3$. The total flavonoid content was determined using a standard curve with quercetin the standard. Total flavonoid content is expressed as mg of quercetin equivalents (QE)/gm of extract [18].

Hydroxyl radical scavenging (OH) assay

Hydroxyl radical scavenging activity was determined by the method of Halliwell *et al.* (1987). Briefly, the reaction mixture, of a final volume of 1.0ml, containing 0.4 ml of 20mM sodium phosphate buffer (pH 7.4), 0.1 ml of 100 -400 μg/ml of methanolic extract, 0.1 ml of 60 mM deoxyribose, 0.1 ml of 10 mM H_2O_2, 0.1 ml of 1 mM ferric chloride, 0.1 ml of 1 mM EDTA and 0.1 ml of 2mM ascorbic acid, was incubated at 37º C for 1hr. Then add 1 ml of 17mM TBA and 1 ml of 17 mM trichloroaceticacid (TCA). The mixture was boiled for 15 min, cooled in ice, and the absorbance measured at 532 nm. Ascorbic acid was used as a positive control. Distilled water in place of test extracts or ascorbic acid was used as control and the sample solution without deoxyribose as sample blank.

Ferric reducing antioxidant power (FRAP) assay

The reductive potential was determined according to the method of Oyaizu (1986) based on the chemical reaction of Fe^{3+} to Fe^{2+}. To 100 – 400 μg/ml methanolic extract and ascorbic acid standard in 1ml of methanol, 2.5 ml each of phosphate buffer (0.2M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (1% w/v) was added and the mixture incubated at 50ºC for 20 min, followed by addition of 2.5 ml of TCA(10% w/v). The mixture was centrifuged for 10 min at1000g, the upper layer (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1% w/v), and the absorbance was measured at 700 nm.

2.5 RESULTS AND DISCUSSION
Preliminary phytochemical analysis

The Preliminary phytochemical screening of the extracts reported the presence of Alkaloids, carbohydrates, flavonoids, saponins, phenols, amino acids, proteins and phytosterols.

Fig.1: Cotton pellet granuloma induction in rat, showing Control, standard Dexamethasone (0.5 mg/kg), MEAC 200 mg/kg, MEAC 400 mg/kg.

The results obtained from the present study provided evidence that MEAC possessed an anti-inflammatory activity in both acute and chronic inflammatory models. In the present study it was demonstrated that p.o. administration of MEAC at the doses of 200 and 400 mg/kg significantly produced dose-dependent inhibition of paw oedema induced by carrageenan and dextran in rats over a period of 3 and 2hrs.respectively, with percentage of inhibition at 3 & 2 h post carrageenan injection of 46.15%, 61.53% & 20.56%, 26.33% (fig 3 & 5) respectively. The carrageenan-induced paw edema formation is a classical model of acute inflammation and it is believed to involve a biphasic event. It is well known that the early phase (1–2 h) is mediated by the release of

histamine and serotonin [19], while the second phase (3–5 h) is the result of the release of kinins and mainly prostaglandins [20]. In general, development of edema induced by carrageenan is correlated with the early exudative stage of inflammation, one of the important processes of inflammation [21]. Based on the present results it can be suggested that the inhibitory effect of MEAC on carrageenan-induced paw edema may be due to the suppression of the release of mediators responsible for inflammation.

The cotton pellet-induced granuloma is an established chronic inflammatory model [22]. The results showed that MEAC at the doses of 200 and 400 mg/kg (p.o.), produced a significant inhibition of 25.63%, 44.97% (fig 7) respectively as compared with Dexamethasone (0.5 mg/kg) which produced significant inhibition of 58.65%. The cotton pellet-induced granuloma has been widely used to evaluate the transudative and proliferative components of chronic inflammation and the dried weight of the pellets correlates well with the amount of granulomatous tissue formed [23].

The antinociceptive effects were assessed using the acetic acid-induced writhing test. MEAC significantly reduced the number of writhing events induced by acetic acid solution at inhibitory rates of 32% and 54%. The standard drug (Ibuprofen) inhibited 56.96% of writhing. These results are shown in Table 4.

The results of the hot-plate test indicate that MEAC (at doses of 200 and 400 mg/kg) significantly increased the latency of the response, which suggests that this extract suppressed the thermal stimulus response.

The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells [24]. Ascorbic acid was highly effective in inhibiting the oxidative DNA damage. The extract displayed potential inhibitory effect of hydroxyl radical-scavenging activity ($IC_{50}=360\mu g/ml$). All results showed hydroxyl radical scavenging activity in dose dependent manner. The results of the FRAP assay indicated that MEAC had stronger reducing power ($IC_{50}=375\mu g/ml$).

Based on the present study, it can be concluded that MEAC possesses both anti-inflammatory and antioxidant activities.
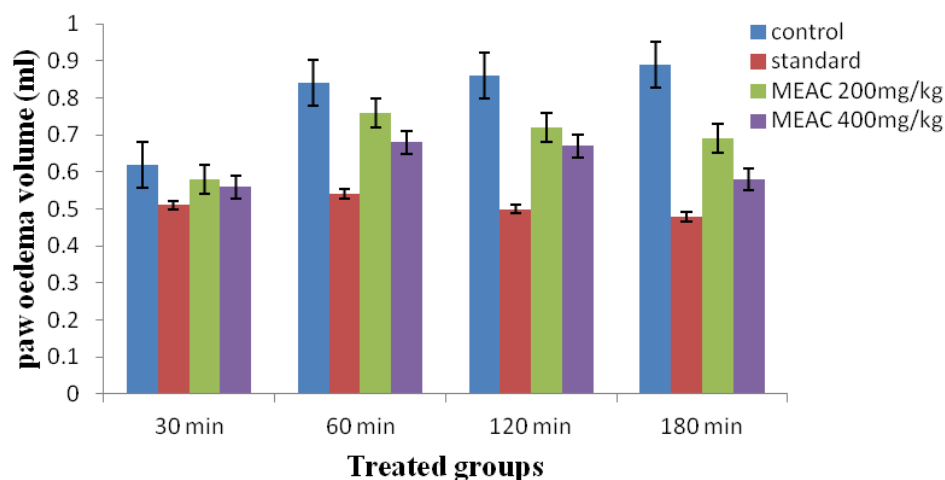
Fig.2 Anti-inflammatory effect of MEAC on Carrageenan-induced rat paw oedema model
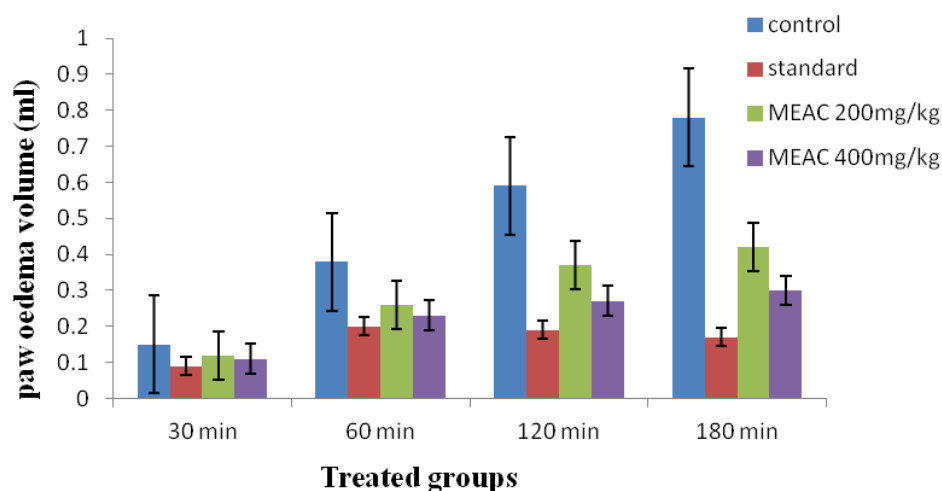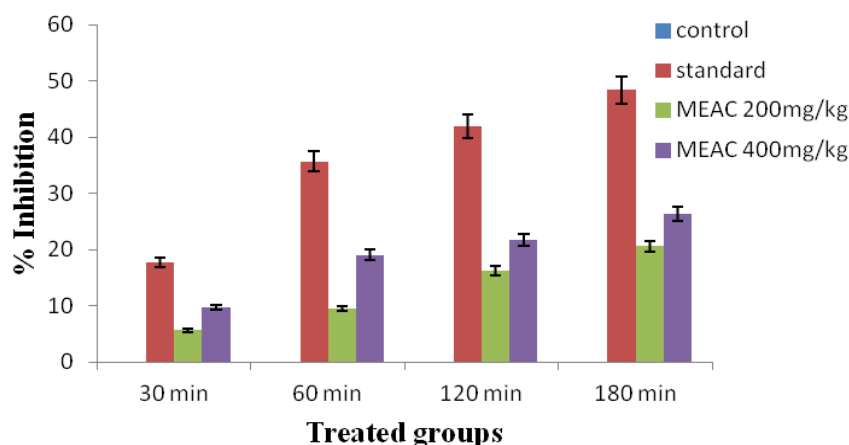
Fig.3: Percentage inhibition of MEAC on Carrageenan-induced rat paw oedema model
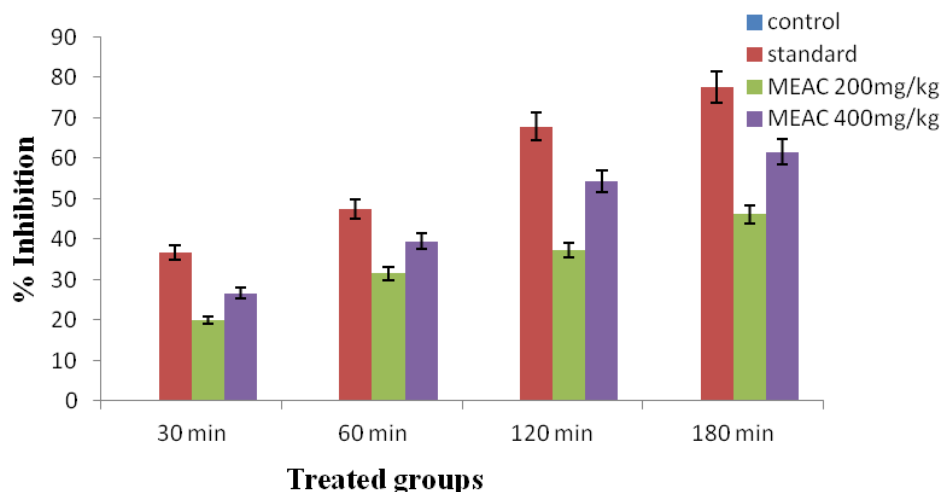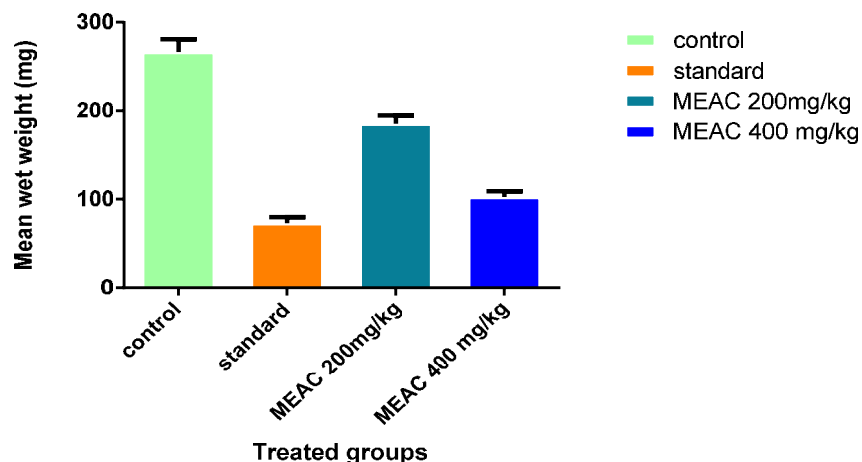

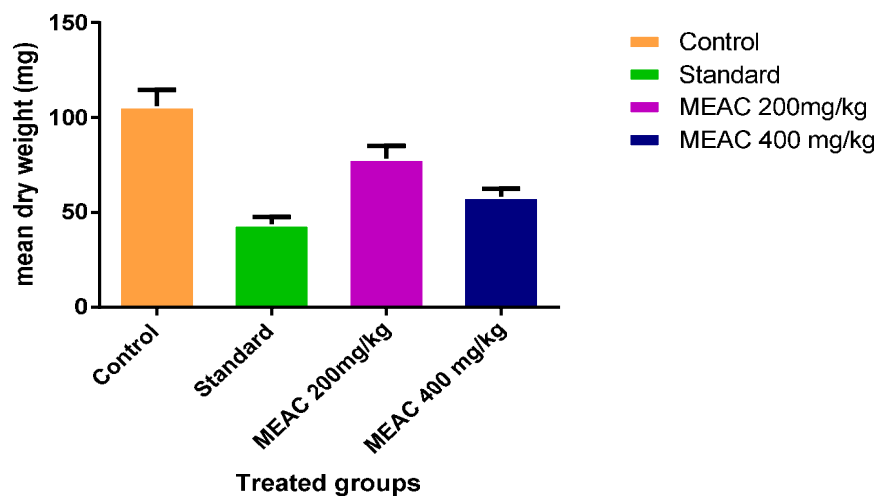**Fig.4:** Anti-inflammatory effect of MEAC on Dextran induced rat paw oedema model.

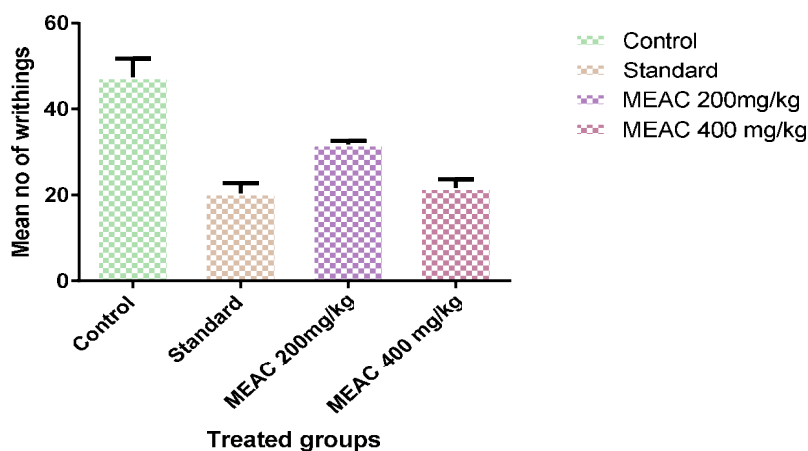**Fig.5:** Percentage inhibition of MEAC on Dextran-induced paw oedema model

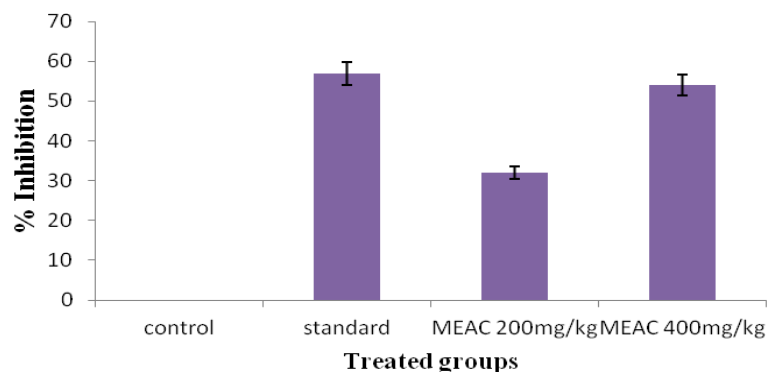**Fig.6:** Effect of the mean wet wt of cotton pellets with respect to different treated groups

Fig.7: Effect of the mean dry wt of cotton pellets with respect to different treated groups
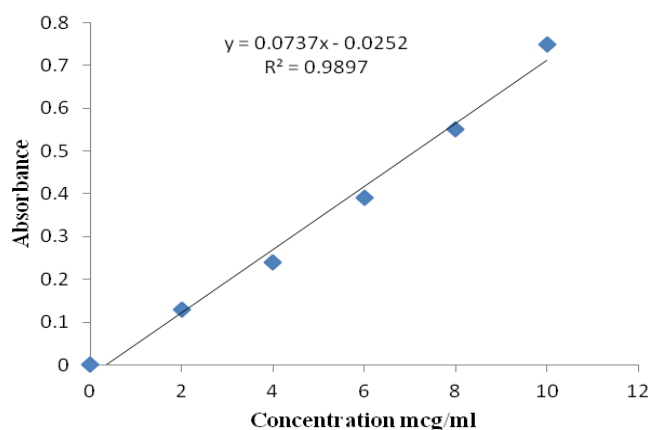

Fig.8: Anti-nociceptive effect of MEAC on Acetic acid induced writhing

Fig. 9: Percentage inhibition of MEAC on Acetic acid induced writhing

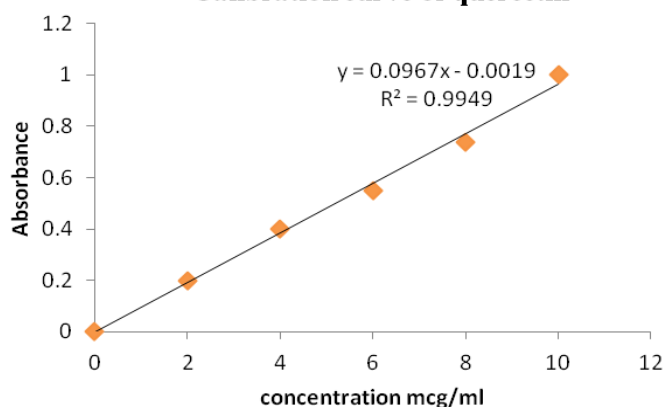## ANTIOXIDANT ACTIVITY:

Total phenolic content

The standard graphs of catechol yielded curve with regression coefficient, r^2=0.989

Fig.10: Calibration curve of catechol

The total phenolic content in the methanolic extract of the *Agave cantala* was estimated by catechol equivalents. The total phenolic content was found to be 6.5 mg catechol per gm weight of extract.

Total flavonoid content

The standard graphs of quercetin yielded curve with regression coefficient, r^2=0.994.

**Fig.11**: Calibration curve of Quercetin

The total flavonoid content in the methanolic extract of the *Agave cantala* was estimated by Quercetin equivalents. The total flavonoid content was found to be 7.4 mg quercetin per gm weight of extract respectively.

Fig.12: Graphical representation of hydroxyl radical scavenging assay
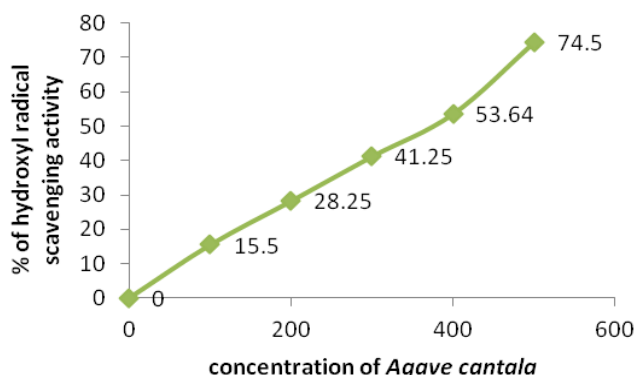
Fig.13: Graphical representation of FRAP assay
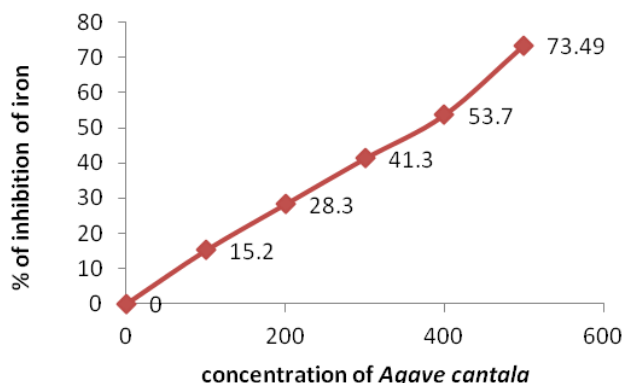
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