Gastro Protective Effect of *Annona muricata* Linn Leaf Extracts in Rats using Pylorus Ligation Method

T Satyanarayana¹, B Gangarao¹, G Surendra¹*, Anjana Male¹

**Abstracts:** The major objective of this study was to evaluate gastro protective effect of hexane extract, ethyl acetate extract, ethanolic extract of *Annona muricata* Linn at dose of 200 mg, 100 mg per kg b.w., was administered orally twice daily for 5 days using pylorus-ligation ulcer model in wistar albino rats. Estimation of various gastric fluids such as volume of gastric juice, total acidity, Free acidity, pH values were done. Ulcer index and percentage protection were determined, all the results obtained were compared with the control group and from the results it was found that chloroform extract treated group has shown dose dependent highly significant gastroprotective action. Thus validating the folklore usage of *Annona muricata* as an antiulcer drug.

**INTRODUCTION**

An ulcer is the result of an imbalance between aggressive and defensive factors. On one hand, too much acid and pepsin can damage the stomach lining and cause ulcers. On the other hand (and more commonly), the damage comes first from some other causes, making the stomach lining susceptible to even an ordinary level of gastric acid [¹]. There is wide variety of allopathic drugs are available for treating ulcers that include proton pump inhibitors, Hydrogen receptor antagonists, which reduces the gastric secretion etc. on repeated use of these drugs leads to adverse effects and also relapsing of ulcer occurs hence there is need of finding of herbal remedy for treating the ulcers which have got less side effects greater therapeutic action. Advanced life style, stress, usage of NSAIDS are the major causes of ulcer induction [²]. The present study is an attempt to evaluate gastro protective action of the *Annona muricata* Linn plant extracts.

*Annona muricata* Linn is widely grown and well cultivated in many areas of Andhra Pradesh. It is commonly called as sour apple/ custard apple which belongs to Annonaceae family, in telugu it is called as laxmanphalamu. Pharmacologically extracts have demonstrated immunostimulatory activity [³]. It is also used in Rheumatism, Nervous diseases, Liver diseases, Diuretic, Dropsy, Cephalalgia, used as awound healing agent [⁴]. It is also used in folklore medicine for treating gastro intestinal disorders [⁵].

**MATERIALS AND METHODS**

**Collection of Specimens**

The leaves of *Annona muricata* Linn were collected from the nearby area of Guntur district fields in February 2011 and was authenticated by prof. D. Ramakanth raju retire botanist and a voucher specimen (T.S.N-001, 12/06 /2011) has been deposited in pharmacognosy department Andhra university.

**Preparation of Plant Extracts**

Collected plant material has been dried under shade and made into coarse powder passed through sieve # 20 and has been successively soxhleated [⁶] using solvents like hexane, ethylacetate and ethanol for 72 hrs. Obtained extracts were made solvent free using rota evaporator and stored in vacuum desiccator. Yield was found to be 9%, 15.5% and 22.3% respectively. Obtained extracts were tested for preliminary phytochemical screening [⁷]. Oral suspensions of the extracts were prepared at a dose of 200 mg/ml and 100 mg/ml using 5% aqueous gum acacia.

**Acute Toxicity Studies**

Adult swiss albino mice 20-25gm were taken for acute toxicity tests. The mice were divided into control and test groups containing 6 animals each. The control group receive vehicle (5%of normal saline) and the test group receive graded doses of extracts. The animals were observed carefully up to 4 hours then occasionally up to 48 hours for sign of any behavioural changes and motility and LD 50 values were calculated [⁸].

**Determination of Gastro Protective Activity**

The experimental protocol was approved by the animal ethical committee of Andhra university, Visakhapatnam, which was registered with the committee for the purpose of control and supervision of experiments on animals (CPCEA), govt of India (registration no 516/01/A/CPCEA)

**Selection of Animals** [⁹]

Wistar albino rats weighing 150-200gm of either sex were used. The animals were fed with balanced diet and tap water *ad libitum*. The animals were maintained at room temperature and 40-70% RH with 12hr light period (6:00-18:00).

**Drug Protocol** [¹⁰]

Animals are divided into eight groups, each group consists six rats. Group I Control group were received acacia suspension 1mg/kg b.w orally. Group II received standard drug omeprazole 20mg/kg b.w. a reference drug for ulcer protective studies [¹¹] Group III received Hexane extract of 100mg/kg, Group IV received Hexane extract of 200mg/kg body weight, Group V received Ethyl acetate extract of 100mg, Group VI received ethyl acetate extract 200mg/kg, Group VII and VIII received ethanolic extract of 100, 200mg/kg b.w respectively, twice daily for 5 days.
Pyloric Ligation in Rats

On 5th day after 45 min of extracts and Omeprazole treatment, pyloric ligation [12] was done by ligating the pyloric end of stomach of rats of respective groups under light ether anaesthesia [13] at a dose of 35 mg/kg of body weight. Ligation was done without causing any damage to the blood supply of the stomach. Animals were allowed to recover and stabilize in individual cages and were deprived of water during postoperative period. After 4 hrs of surgery, rats were sacrificed and ulcer scoring [14] was done. Gastric juice was collected and gastric secretion studies were performed [15]. Gastric contents were analyzed for total acidity by titrating against 0.01N NaOH [16] using phenolphthalein as indicator.

The pH of the gastric juice was measured by using pH paper strips of varying ranges. The colour of pH paper after the procedure was matched with the standard scale and pH was recorded [17].

Scoring of Ulcer will be Made as Follows

- Normal stomach…..(0)
- Red coloration…..(0.5)
- Spot ulcer..........(1)
- Hemorrhagic streak…..(1.5)
- Ulcers.............(2)
- Perforation..........(3)

Mean ulcer score for each animal will be expressed as ulcer index.

The percentage of ulcer protection was determined as follows:

% protection = \frac{\text{Control mean ulcer index} - \text{Test mean ulcer index}}{\text{Control mean ulcer index}} \times 100

Values are represented as mean ± S.E.M for 6 rats.
Analysis of variance (ANOVA) followed by Dunnet's test was done and the P-value <0.05 were considered statistically significant for the experiment.

RESULTS AND DISCUSSION
This work was an attempt made for the validation of rational usage of Annona muricata Linn as a gastroprotective agent. In acute toxicity study no mortality was found up to 1000mg/kg p.o of Annona muricata plant extracts treated animal group. The LD$_{50}$ was determined and 1/10$^\text{th}$ and double of 1/10$^\text{th}$ doses of the tested proven safe concentrations were taken as our experimental dose.

Preliminary phytochemical tests of the extracts shown in Table 1, Effect of different extracts of annona muricata Linn on pylorus ligated ulcers model in rats shown in Table 2 and Effect of different extracts of A. muricata on pylorlc ligation method on induction of ulcer in rats and %protection of the extracts: shown in Table 3.

Discussion
In the past, it was believed stress and diet caused peptic ulcers. Later, researchers stated stomach acids (hydrochloric acid and pepsin) contributed to the majority of ulcer formation [18]. Today, however, research shows that most ulcers develop as a result of infection with a bacterium called Helicobacter pylori [19]. Pylorus ligated ulcers caused by enhanced acid pepsin secretion leading to autodigestion of gastric mucosa and break down gastric mucosal barrier [20]. The digestive effect of accumulated gastric juice results in interference with gastric blood circulation and responsible for induction of ulcers [21]. All the results of the extracts were compared with the standard drug treated group at a dose of 20mg/kg b.w. Pretreatment with the ethyl acetate extract of annona muricata Linn at a dose of 200mg/kg b.w significantly reduced the gastric juice volume and at the same time it has significantly raised the pH showing highly significant action, where as ethanolic extract also decreased total acidity and increase pH showing moderate significant, hexane extract at a dose of 200mg average significance it was showing. The percentage protection against the ulcer was shown by standard drug omeprazole at 72%, 69% was shown by ethyl acetate extract a dose of 200 mg. kg b.w, 60% protection was shown by ethyl acetate extract a dose of 50mg/kg b.w, hence it was exhibiting dose dependent activity. Ethanol extract exhibited the protection of 61%
and 56% at a dose of 100mg/kg and 50mg /kg b.w respectively. petroleum ether extract was exhibiting lesser protection of 55 % and 46% at a dose of 100mg and 50mg/kg b.w respectively.

Form this work we conclude that the folklore usage of annona muricata as gastro protective agent has been validated . Chloroform extract of Annona muricata Linn has shown high protection against ulcer this could be due to the presence of mucilage, steroids and alkaloids [22].

REFERENCES AND NOTES

Acknowledgments
Authors are thankful for the help and support provided by the krishnateja pharmacy college in fulfilment of the work.

PHARMACOGNOSTICAL AND PHYTOCHEMICAL STUDIES OF

ANNONA RETICULATA LINN

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ABSTRACT
Plants have been one of the important sources of medicines since the beginning of human civilization. There is a growing demand for plant based medicines, health products, pharmaceuticals, food supplements, cosmetics etc. *Annonareticulata Linn* is a multipurpose tree with traditional uses as an antioxidant, antidiabetics, hepatoprotective, cytotoxic activity, genotoxicity, antitumour activity, antilice agent. It is related to contain alkaloids, carbohydrates, fixed oils, tannins & phenolic. To supplement the necessary information for the systematic identification and authentication of this particular species, pharmacognostic standardization, macroscopic, microscopical study of various parts of this plant as per WHO guidelines and phytochemical studies on various crude extracts obtained from the leaf extracts were carried out using three different polar solvents and the results were reported.

Keywords: *Annonareticulata*, pharmacognostical standardization, physicochemical studies.

INTRODUCTION
According to the WHO survey 80% populations living in the developing countries rely almost exclusively on traditional medicine for their primary health care needs. Exploration of the chemical constituents of the plants & pharmacological screening may provide us the basis for developing the leads for development of novel agents. In addition, herbs have provided us some of the very important life saving drugs used in the modern medicine. However among the estimated 250,000-400,000 plant species, only 6% have been studied for biological activity and about 15% have been investigated phytochemically¹,². *Annonareticulata Linn* is a small ever green tree is cultivated throughout India for its fruits, differentparts of *Annonareticulata Linn*. are used in folkloric medicine for the treatment of variousdisease³. This plant is commonly called custard apple in English & ramaphalam in telugu in India⁴. *Annonareticulatalinn* is a shrub or small tree 7 m height & is cultivated throughout India. To ensure reproducible quality of herbal products, authentication of the starting material is essential. According to WHO (1988), 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.

*Annonareticulata Linn*, belonging to family *Annonaceae* is commonly found in India & cultivated in Thailand & originates from the WestIndies & south America. It is mainly grown in gardens for its fruits & ornamental value. It is known as custard apple, sugar apple, sweet apple in English, ramaphalam in Telugu in India⁵. A root decoction is taken as a febrifuge, while fragments of the root bark are packed around the gums to relieve toothache. The bark
is very astringent and the decoction is taken as a tonic and also as a remedy for diarrhea and dysentery. The leaf decoction is given as a vermifuge. Crushed leaves or a paste of the flesh may be poulticed on boils, abscesses and ulcers. The unripe dried fruit dried is employed against diarrhea and dysentery. In severe cases, the leaves, bark and green fruits are all boiled together for 5 minutes in a liter of water to make an extremely potent decoction. (Morton, J. 1987. Fruits of Warm Climates.)

**Fig. 1: leaf and fruit of Annonareticulata**

**Collection of Specimens**
The leaves of *Annonareticulata* Linn were collected from the nearby area of tirumala hills in August 2010 and were authenticated by prof. D. Ramakanthraju retirerobotist and a voucher specimen (T.S.N-005, 12/08 /2010) has been deposited in pharmacognosy department Andhrauniversity.

Care was taken to select healthy plants and for normal organs. The leaf, stem, root and stem bark were cut and removed from the plant and fixed in formalin acetic acid solution (Formalin:acetic acid:70% ethyl alcohol in the ratio of 0.5:0.5:9). After 24 h of fixation, the specimens were dehydrated with graded series of tertiary-Butyl alcohol (Sass J E; 1940). Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58 - 60°C) until thiobarbituric acid solution attained super saturation. The specimens were then casted out into paraffin blocks.

**MATERIALS AND METHODS**

**Preparation of sections**
The paraffin embedded specimens were sectioned with the help of a rotary microtome. 10 - 12 µm thickness of the sections was made. However, dewaxing of the sections was done using customary Procedure. The sections were later stained with toluidine blue. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. For the study of isolated cells leaves were macerated with concentrated nitric acid and potassium chloride, washed with distilled water and mounted in glycerine, qualitative and quantitative microscopic evaluation was done along with Ultraviolet fluorescence analysis of powdered drug.

Collected leaf powder was subjected to preliminary and microbiopical examination, physicochemical evaluations which include ash value, acid insoluble and water soluble ash, extractive value (hexane soluble, ethyl acetate soluble and ethanol soluble) and moisture contents (loss on drying), swelling index, foaming index were determined.

**Photomicrographs**
Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photomicrographs of different magnifications were taken with electron microscopic unit of quantum model. For normal observation, a bright field microscopy was used and for the study of crystals, starch grains and lignified cells, a polarized light was employed. However, since these structures have birefringent property, under polarized light they tend to appear bright against the dark background.

**RESULTS AND DISCUSSION**

**Morphological study**

*Annonareticulata* is a small, semi-deciduous tree, 3-7m in height, with a broad, open crown or irregularly spreading branches, bark light brown with visible leafscars and smoothish to slightly fissured into plates.

**Inner bark**: light yellow and slightly bitter

**Twigs**: Become brown with light brown dots.

**Leaves**: Occurringly, 6-17 x 3-6 cm, lanceolate or oblong lanceolate, pale green on both surfaces and glabrate or nearly so. Sides sometimes slightly unequal, edges without teeth, inconspicuously hairy, at least when young minutely dotted on examination with a lens, thin, dullgreen to dark green on top surface, and pale blue-green and covered with bloom on underside; apex short or long pointed. Base short pointed or rounded.
Petioles: 0.6-1.3 cm long, green, sparsely pubescent.

Flowers: greenish-yellow, fragrant, on slender hairy stalks. Produced singly or in short lateral clusters about 2.5 cm long, 2-4 flowers but not at the base of the leaves; sepals pointed, hairy, green, about 16 mm long, 3 outer petals oblong, thick and rounded at the tips, fleshy, 1.6-2.5 cm long, 0.6 cm wide, yellow-green, slightly hairy inside light yellow and keeled with a purplish or reddish spot at the thin, enlarged base. Inner petals 3 minute, ovate, pointed scales.

Stamens: very numerous, crowded, white, less than 16 mm long.

Ovary: light green,

Styles: white, crowded on the raised axis.
The aggregate fruit formed from the numerous pistils of a flower, which are loosely united, is soft and distinct from other species of the genus. Each pistil forms a separate tubercle, mostly 1.3-1.9 cm long and 0.6-1.3 cm wide. Fruit is round, heart shaped, ovate or conical, 5-10 cm in diameter, with many round protuberances; greenish-yellow when ripe, with a white, powdery bloom. The pulp is white, edible and sweetly aromatic.

In each carpel is embedded a seed, oblong, shiny and smooth, blackish or dark brown, 1.3-1.6 cm long, numerous 3-4 (fig. 1)

T.S of leaf: Transverse section through midrib shows the upper and lower single layered compactly arranged rectangular to barrel shaped epidermis with thick cuticle and multicellular trichomes filled with tannin on lower surfaces. Lamina upper single layered palisade parenchyma and lowers 6-7 layers of spongy parenchyma lysogenous cavities are very common, prismatic crystals, oil globules and tannin content material spread throughout the lamina and also even in midrib. Through midrib shows vascular bundle radially arranged. Vascular bundle surrounded by pericyclic fibres on both the side, rest of consist parenchyma cells, in center a group of stone cells is observed (fig. 2) surface study the upper and lower epidermis of the leaf was peeled off and observed under the microscope, the upper epidermis show only epidermal cells and lysogenous cavity and oil globules where as lower epidermis shows paracytic stomata epidermis cells, lysogenouscavity, oil globules (fig. 2)

Powder microscopy
powder shows paracytic stomata from lower surface, fragment of fibers with narrow lumen, multicellular trichome filled with tannin content from epidermal surface, microrosette crystals of calcium oxalate pitted stone cells with wide lumen, annular vessels from vascular bundle (fig. 3)
Transverse section of stem
It shows collenchymatous cells below epidermis, followed by pericyclic fibers, xylem, phloem, and parenchymatous cells. Xylem is surrounded by starch grains and pith contains lignified stone cells. Starch grains are oval or ellipsoid, turning blue when treated with iodine. Transverse section of stem bark showed the presence of 7-8 layers of uniformly arranged cork cells followed by cortex cells, it also contains radially dividing parenchyma cells. Also contains wood elements, lignified fibers.

Transverse section of root shows wide cortex made up of a group of stone cells, the phloem shows large sieve tubes interspersed with phloem parenchyma and fibres. The cell types and cell inclusion are detected in the powdered root bark.

T.S of Annona reticulata stem bark

T.S of Annona reticulata stem

Determination of quantitative microscopic evaluation of Annonareticulata leaf
Stomatal index, stomatal number, vein islet, vein termination number was determined for the peeled of Annonareticulata leaf and the results were given in the Table 1.

Determination of physicochemical parameters of Annonareticulata leaf
Fresh materials of A. reticulata leaves, were collected and subjected to various physicochemical parameters such as moisture content were observed and recorded. Ash values are helpful in determining the quality and purity of crude drug, especially in the powder form. Total ash reflects the care taken in its preparation as all traces of organic matters were removed during ash formation and usually consists of carbonates, phosphates and silicates.
of sodium, potassium, calcium and magnesium. (Table 2)

<table>
<thead>
<tr>
<th>Leaf constants</th>
<th>A. reticulata</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomatal number upper</td>
<td>164-178</td>
</tr>
<tr>
<td>Lower</td>
<td>182-196</td>
</tr>
<tr>
<td>Stomatal index upper</td>
<td>16.3-19.5</td>
</tr>
<tr>
<td>Stomatal index lower</td>
<td>22.5-26.4</td>
</tr>
<tr>
<td>Palisade ratio</td>
<td>9-11</td>
</tr>
<tr>
<td>Vein islet number</td>
<td>8.5-10.6</td>
</tr>
<tr>
<td>Vein termination number</td>
<td>11.3-16.4</td>
</tr>
</tbody>
</table>

Table 2: Results of physicochemical properties

<table>
<thead>
<tr>
<th>Physicochemical properties</th>
<th>A. reticulata Value in %w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total ash</td>
<td>16.5</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>16.1</td>
</tr>
<tr>
<td>Water soluble ash</td>
<td>7.5</td>
</tr>
<tr>
<td>Extractives values hexane extract</td>
<td>5.5</td>
</tr>
<tr>
<td>Ethylacetate extract</td>
<td>18.2</td>
</tr>
<tr>
<td>Alcohol extract</td>
<td>19.3</td>
</tr>
<tr>
<td>L.o.d</td>
<td>5</td>
</tr>
<tr>
<td>F.o.m</td>
<td>2.1</td>
</tr>
<tr>
<td>Swelling index</td>
<td>6</td>
</tr>
<tr>
<td>Foaming index</td>
<td>-</td>
</tr>
<tr>
<td>Volatile oil content</td>
<td>0.0</td>
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Table 3: Analysis of leaf powder of Annonareticulatalinn

<table>
<thead>
<tr>
<th>s.no</th>
<th>Reagents with powder</th>
<th>Daylight</th>
<th>Short wave length</th>
<th>Long wave length</th>
</tr>
</thead>
<tbody>
<tr>
<td>1)</td>
<td>Leaf powder</td>
<td>Green</td>
<td>Light green</td>
<td>Dark green</td>
</tr>
<tr>
<td>2)</td>
<td>Powder+water</td>
<td>Dark green</td>
<td>Brown</td>
<td>Brownish red</td>
</tr>
<tr>
<td>3)</td>
<td>Powder+ethanol</td>
<td>Dark brown</td>
<td>Light red</td>
<td>Dark red</td>
</tr>
<tr>
<td>4)</td>
<td>Powder +dilHCl</td>
<td>Light brown</td>
<td>Light brown</td>
<td>Light brown</td>
</tr>
<tr>
<td>5)</td>
<td>Powder + dil H2SO4</td>
<td>Dark brown</td>
<td>Dark brown</td>
<td>Dark brown</td>
</tr>
<tr>
<td>6)</td>
<td>Powder + dil HNO3</td>
<td>Red</td>
<td>Orange red</td>
<td>Reddish orange</td>
</tr>
<tr>
<td>7)</td>
<td>Powder + aq.NaOH</td>
<td>Dark green</td>
<td>Dark brown</td>
<td>Dark green</td>
</tr>
<tr>
<td>8)</td>
<td>Powder + alc.NaOH</td>
<td>Dark green</td>
<td>Light Red</td>
<td>Dark Red</td>
</tr>
<tr>
<td>9)</td>
<td>Powder + aq.KOH</td>
<td>Light green</td>
<td>Light green</td>
<td>Light green</td>
</tr>
<tr>
<td>10)</td>
<td>Powder + alc.KOH</td>
<td>Green</td>
<td>Light brown</td>
<td>Darkbrown</td>
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</table>

Table 4: Analysis Of Extracts Annonareticulata Linn

<table>
<thead>
<tr>
<th>s.no</th>
<th>Extract</th>
<th>Nature of extract</th>
<th>Appearance in Day light</th>
<th>Short wave length</th>
<th>Long wave length</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hexane extract</td>
<td>Semi solid</td>
<td>Dark green</td>
<td>Light green</td>
<td>Dark red</td>
</tr>
<tr>
<td>2</td>
<td>Ethyl acetate extract</td>
<td>Semi solid</td>
<td>Light green</td>
<td>Light green</td>
<td>Dark red</td>
</tr>
<tr>
<td>3</td>
<td>Alcohol extract</td>
<td>Semi solid</td>
<td>Greenish brown</td>
<td>Dark brown</td>
<td>Reddish brown</td>
</tr>
</tbody>
</table>

Table 5: results of phytochemical tests of various leaf extracts of Annonareticulatalinn

<table>
<thead>
<tr>
<th>s.no</th>
<th>Test</th>
<th>Hexane extract</th>
<th>Ethyl acetate extract</th>
<th>Ethanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Aminoacids</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Carbohydrates</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Mucilage</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Proteins</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Starch</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Steroids&amp;triterpenoids</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Glycosides</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Preliminary phytochemical studies (Table 5)
The qualitative chemical investigation of all the extractsof selected plant was carried out to check the presence of various phytoconstituents. It revealed the presence of steroids in hexane extract. Ethylacetate extract revealed the presence of alkaloids, carbohydrates, flavonoids, mucilage. Where as ethanolic extract revealed the presence of alkaloids, carbohydrates, mucilage, glycosides.

CONCLUSIONS
Medicinal plants are valuable natural sources and regarded as potential and safe drugs. They have been playing an important role as natural drugs to alleviate humansufferings by contribution herbal medicines to the primary
health care systems of rural and remote areas where more than 70% of population in India depend on folklore and traditional systems of medicines\textsuperscript{11}. From the pharmacognostic, and phytochemical investigations, it is quite possible to set the standards of this plant as per the pharmacopoeial guidelines and it will be use full for selecting the proper herb and for the research to carry out.

REFERENCES
INTRODUCTION
Anthelmintics or antihelminthics are the drugs or the agents that destroy or cause the expulsion of parasitic intestinal worms. Helminthiasis is a macroparasitic disease of humans and animals in which a part of the body is infested with parasitic worms such as pinworm, roundworm, or tapeworm. It can have immunomodulatory effects on the host, with implications for any co-infecting pathogens. More than half of the population of the world suffers from infection of one or the other and majority of cattle’s suffers from worm infections. Gastrointestinal parasitism of sheep and goats is one of the leading causes of mortality, producing high economic losses. This infection can be controlled with chemical medicinal agents but improved management is the most important infection control strategy throughout the world. Chemical control of helminths coupled with improved management has been the important worm control strategy throughout the world. However, increasing problems of development of resistance in helminths against anthelmintics have led to the proposal of screening medicinal plants for their anthelmintic activity. The plants are known to provide a rich source of botanical anthelmintics. A number of medicinal plants have been used to treat parasitic infections in man and animals. Albendazole is the first drug of choice for the treatment of worm infections. It is also first reported anthelmintic which promises to have useful activity against all the types of helminth parasites menacing the domestic animals. We have focused our attention on search of herbal remedy and selected Annona squamosa, the sugar apple that grows in tropical countries. Literatures of many research works prove that every part of A. squamosa possess medicinal property. Roots are employed internally in spinal diseases. Bark is known to be a powerful astringent. In Ayurveda, fruits are considered as a good tonic; enriches blood, used as expectorant, increases muscular strength; cooling, lessions burning sensation and tendency to biliousness; sedative to heart and relieves vomiting. Ripe fruit along with salt is used against malignant tumors to hasten suppuration. Dried unripe fruit is powdered and mixed with gram-flour to destroy vermin. The seeds are said to be abortifacient and good to destroy lice in hair in unani medicine. Seed yields oil and resin which acts as detergent and their powder, is mixed with gram-flour, is a good hair wash. Seeds are powerful irritant of conjunctiva and produce ulcers in the eye. Leaves are used as poultice over boils and ulcers and also to kill lice. It is also reported that 5% (w/w)

ABSTRACT
The aim of the present study was to investigate the anthelmintic activity of the Annona squamosa (Annonaceae), leaf extract using adult earthworm, Peritima posthuma. The hexane, ethylacetate, ethanolic extracts of the crude drug at concentrations of 100mg/ml, 200mg/ml, were tested which involve determination of paralysis time and death time. Albendazole (10mg/ml) was used as standard and it was found that the Annona reticulata leaf extracts showed dose dependent activity ethyl acetate extract at a dose of 200mg/ml has shown very high significant action.

Keywords: Anthelmintic activity, Albendazole, Earthworm, Annona squamosa.
ointment of alcoholic extract of dried leaves in white petroleum jelly is used in wound healing\textsuperscript{15}.

**MATERIAL AND METHODS**

**Collection of Plant Materials**
The leaves of *Annona squamosa* were obtained from tirumala forest area which were authenticated by Mr. D. Ramakanth raju retire botanist.

**Preparation of extracts**
Obtained leaves were shade dried and made into coarse powder and extracted successfully using various polar grade solvents like hexane, ethylacetate, ethanol. Obtained extracts were made solvent free using rota evaporator. Extracts were stored in dessicator until further use.

**Experimental Model**
Adult earthworm *Peritima posthuma* were collected (due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human being\textsuperscript{16, 17} from moist soil, obtained from agricultural fields nearby agricultural field.

Three test groups were taken each containing six earth worms of approximately equal size (8±1 cm). Albendazole was taken as standard drug and different concentrations (10mg/ml) were prepared in normal saline containing 5% DMF\textsuperscript{18-20}. The *Annona squamosa* leaf extracts of different concentrations were prepared by dissolving in minimum quantity of DMF and making up to the final volume with normal saline to obtain 100mg/ml, 200mg/ml. One of the groups is taken as control group which was treated with normal saline containing 5% DMF. Paralysis onset time and death time of individual worms were noted. Paralysis was said to occur when the worms do not retrieve even in normal saline. Death was concluded when the worms lost their motility followed by fading away of color of worm.

**RESULTS AND DISCUSSION**
The data in Table-1 reveals that the *Annona squamosa* Linn leaf extracts showing significant anthelmintic activity compared to the standard.

<table>
<thead>
<tr>
<th>s.no</th>
<th>Treatment</th>
<th>Dose</th>
<th>Paralysis time in mins</th>
<th>Death time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>5% DMF in saline solution</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Albendazole</td>
<td>10mg/ml</td>
<td>2.55±0.76</td>
<td>4.37±0.66</td>
</tr>
<tr>
<td>3</td>
<td>Hexane extract</td>
<td>100mg/ml</td>
<td>25.56±0.56</td>
<td>27.62±0.14</td>
</tr>
<tr>
<td>4</td>
<td>Hexane extract</td>
<td>200mg/ml</td>
<td>21.43±0.64</td>
<td>23.11±0.26</td>
</tr>
<tr>
<td>5</td>
<td>Ethyl acetate extract</td>
<td>100mg/ml</td>
<td>4.27±0.02</td>
<td>6.43±0.07</td>
</tr>
<tr>
<td>6</td>
<td>Ethyl acetate extract</td>
<td>200mg/ml</td>
<td>2.19±0.11</td>
<td>4.11±0.09</td>
</tr>
<tr>
<td>7</td>
<td>Ethanol extract</td>
<td>100mg/ml</td>
<td>12.43±0.88</td>
<td>16.12±0.45</td>
</tr>
<tr>
<td>8</td>
<td>Ethanol extract</td>
<td>200mg/ml</td>
<td>9.16±0.66</td>
<td>12.17±0.16</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM from six observations;
Control worms were alive up to 24 hrs of observation.
From the results we can find Annona squamosa Linn leaf extracts showed dose dependent activity, out of all three extracts ethylacetate extract at a dose of 200mg/ml showed high significant action by killing worm in 4.11±0.09 mins. Ethanolic extract showed significant by killing the worm in 12.17±0.16 mins. Hexane extract showed moderate significant action. Standard drug albendazole showed death of worm in 4.37±0.66 mins. Ethyl acetate extract has shown equal to standard drug albendazole.

CONCLUSION
From the work we conclude that traditional usage of Annona squamosa leaf for treating worm infestations has been validated.

REFERENCES
Decision on Manuscript: D-5156: The Journal of Free Radicals and Antioxidants

Photon Journal <submissionsphoton@gmail.com> Wed, Apr 17, 2013 at 12:27 PM
To: grandhi.surendra@gmail.com, Photon Journals <photonjournals@gmail.com>

Decision on Manuscript

Dear Dr. G.Surendra

Welcome at Photon. We have received a Detailed Peer Reviewed Report on your Manuscript ID: D-5156 Entitled as: In vitro Antioxidant potential screening of different leaf extracts of Annona reticulata Linn

Authored by: Dr.B. Gangarao, Dr.T.satyanarayana, G.Surendra*, G.Gowrisankar, M.Vani

from panel of eight international reviewers from USA, Canada, Germany, France, UK, Venezuela, Japan and Australia. The said matter has been placed in the meeting of Board of Editors held on 16 April, 2013. It is decided that Manuscript ID: D-5156 can be published on The Journal of Free Radicals and Antioxidants.

Manuscript ID: D-5156 has been transferred to Publication Cell, Photon. You are required to acknowledge us the payment as below so as to receive the Galley Proof of Manuscript ID: D-5156 along with Reviewer’s Recommendations at photonjournals@gmail.com, and submissionsphoton@gmail.com (both IDs).
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Date : 06/05/2013  
Abstr. no. : 77  
Concerns : FIP World Congress 2013  

Abstract : In vitro Anti oxidant potential screening of leaf extracts of Annona reticulata Linn  

Dear Mr Grandhi,  

We have the pleasure of informing you that the above abstract has been accepted for POSTER presentation during the FIP World Congress 2013, which will be held in Dublin, Ireland, from 31 August - September 5, 2013. Please read the instructions below.  

Congress registration required  
Abstracts can only be presented and will only be published if the presenting author has registered and paid for the Congress before 15 May 2013.  
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More detailed information  
The following information will be sent by e-mail and published on the congress website after 1 August  
- Your poster board number  
- Your day(s) of presentation  
- Time slots for hanging up and taking down your poster  

With kind regards,  
On behalf of the Organizing Committee,  

Kind regards,  
Sophie Hamburger  
FIP Congress Secretariat  
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