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Pharmacognostical and Phytochemical Studies on fruits of *Catunaregam spinosa* Linn.

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ABSTRACT

*The aim of the present study was to assess the Pharmacognostic and Phytochemical studies on fruits of *Catunaregam spinosa* Linn. The objective of the present work comprise of collection, identification, microscopical and phytochemical evaluation of fruits of *Catunaregam spinosa* Linn. The fruit sections were taken and cellular structures were studied. The T.S of fruit shows the presence of epidermis, vascular bundle, embryo, placental tissue, septus, and calcium oxalate crystals. The phytochemical studies indicate the Ash value, Extractive value, Crude fibre content, fluorescence characters and the preliminary phytochemical test on various extracts indicates the presence of alkaloids, glycosides, flavanoids, saponins, carbohydrates, tannins, phenols and mucilage.*

Keywords: *Catunaregam spinosa*, Rubiaceae, alkaloid, saponins.

INTRODUCTION

Catunaregam spinosa Linn. (Family-Rubiaceae) is a rigid shrub or tree, upto 9 m in height and 1.2 m in girth found throughout India upto an altitude of 1,900 m. The species is common as an undergrowth in the sub-Himalayan tract and elsewhere. The flowers and fruits are edible, the flowers are eaten as a vegetable in Nasik district. The raw fruits have a highly astringent taste due to high tannin content. The fresh fruits contain high amount of carbohydrate, and saponins. The seeds contain essential oil and organic acid. The fruit pulp dried and powdered is credited with emetic properties. The main uses of *Catunaregam spinosa* are nauseant, expectorant, anthelmintic and abortifacient properties. It also showed hypoglycaemic, piscicidal, insecticidal and anti-

cancer activities. The seeds are tonic to induce appetite. Their decoction is taken for relief from headache. The bark is used to diarrhoea and dysentery.

EXPERIMENTAL SECTION

The plant selected for the present study is *Catunaregam spinosa* Linn. belongs to the family Rubiaceae was collected and authenticated by the botanist Dr.P.Jayaraman, M.Sc., Ph.D. Director Plant Anatomy Research Center (PARC), Tambaram, Chennai.

Microscopical study of the Fruits

Collection of specimens

The plant specimens for the proposed study were taken to select healthy plants and normal organs. The required samples of fruit were cut and removed from the plant and fixed in FAA (Formalin-5ml+ Acetic acid-5ml + 70% Ethyl alcohol-90ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary- Butyl alcohol as per the schedule given by Sass, 1940. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60 C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning:

The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12 μ m. Dewaxing of the sections was by customary procedure (Johansen, 1940). The sections were stained with Toluidine blue as per the method published by O'Brien et al. (1964). Since Toluidine blue is a polychromatic stain, the staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. Wherever necessary sections were also stained with safranin and Fast-green and IKI (for starch) Glycerine mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared with NaOH and mounted in glycerine medium after staining. Different cell component were studied and measured.

Photomicrographs:

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon lab photo 2 microscopic unit. 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. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard Anatomy books (Easu, 1964).

Identification of the plant constituents by phytochemical tests:

The various extracts of drug sample were subjected to chemical test for identification of its active constituents.

Test for Alkaloids:

A small portion of solvent free extracts were stirred separately with few drops of dilute hydrochloric acid and filtered & tested carefully with various alkaloidal reagents.

Mayer's reagent	-	Cream precipitate
Dragendroff's reagent	-	Orange brown precipitate
Hager's reagent	-	Yellow precipitate
Wagner's reagent	-	Reddish brown precipitate

Test for Carbohydrates & Glycosides:

The minimum amount of extracts were dissolved in 5ml of distilled water & filtered. The filtrate was subjected to test for carbohydrates & glycosides.

a)Molisch's test

The filtrate was treated with 2-3 drops of 1% alcoholic alpha naphthol & 2ml concentrated sulphuric acid was added along the sides of test tube. Violet ring was observed at the junction of 2 layers which showed the presence of carbohydrate.

b)Fehling's test

The filtrate was treated with Fehling's A and B. The appearance of reddish brown colour precipitate indicate the presence of reducing sugar.

Test for Glycosides:**a)Legal's test**

The extract was treated with 1 ml of sodium nitroprusside solution & then it was made alkaline with sodium nitroprusside. Pink colour was produced and it indicate the presence of glycosides.

b)Borntrager's test

Hydrolysate was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. No colour change in ammonical layer was observed.

Test for Phytosterol:

1 g of different extracts were dissolved in few drops of dilute acetic acid; 3ml of acetic anhydride was added followed by few drops of concentrated sulphuric acid. Appearance of bluish green colour showed the presence of phytosterol.

Test for Fixed Oil & Fats:

A) Small quantities of various extracts were separately pressed between two filter papers. No oil stain on the paper indicated the absence of fixed oil.

b) Few drops of 0.5N alcoholic potassium hydroxide were added to small quantity of various extracts along with a drop of phenolphthalein. Mixture was heated on water bath for 1-2 hours. No soap formation, neutralization of alkali indicated the absence of fixed oil and fats.

Test for Lignin :

With alcoholic solution of phloroglucinol and hydrochloric acid the appearance of red colour showed the presence of lignin.

Test for Saponins :

The extracts were diluted with 20ml of distilled water and it was agitated on graduated cylinder for 15 minutes. The presence of saponins was indicated by formation of 1cm layer of foam.

Test for Tannins and Phenolic Compounds :

Small quantities of various extracts were taken separately in water and tested for presence of phenolic compounds and treated with

- 1) Dilute ferric chloride solution (5%) - violet colour
- 2) 1% sodium gelatin containing 10% sodium chloride - white precipitate
- 3) 10% lead acetate solution – white precipitate

Test for Proteins and Free Amino Acid:

Dissolved small quantities of various extracts in few ml of water and treated with.

1. **Millon's reagent** – Red colour showed the presence of proteins and free amino acids
2. **Ninhydrin reagent** – Purple colour showed the presence of proteins and free amino acids.
3. **Biuret test** – Equal volume of 5% solution and 1% copper sulphate solutions were added. Appearance of purple colour showed the presence of proteins and free amino acids.

Test for Gums and Mucilage:

Powdered drug was treated with ruthenium red solution. No characteristic colour change was obtained indicating absence of gums & mucilage.

Test for Flavonoids :

- a) With aqueous sodium hydroxide solution - blue to violet colour (Anthocyanins); yellow colour (Flavones); yellow to orange colour (Flavonones)
- b) With concentrated sulphuric acid – yellowish orange colour (Anthocyanins); yellow to orange colour (Flavones); orange to crimson (Flavonones)

Shinoda's test :

Test extracts were dissolved in alcohol, to that piece of magnesium turnings followed by concentrated hydrochloric acid were added drop wise and heated. Appearance of magenta colour showed the presence of flavonoids.

RESULTS AND DISCUSSION

Macro and microscopic characters

The plant is a rigid shrub or tree, up to 9 m in height and 1.2 m in girth found throughout India upto an altitude of 1,900 m. Flowers solitary rarely 2-3 on a peduncle, white later turning yellow fragrant; berry globose or ovoid, 2.0-3.8 cm long, yellow fleshy; seeds flat 4mm long angular.

fig.1



fig.2



fig.3

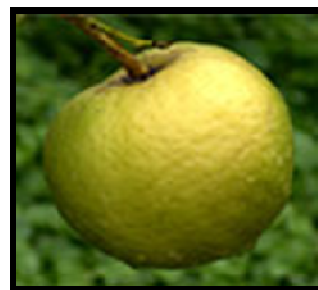


fig.4



fig.5

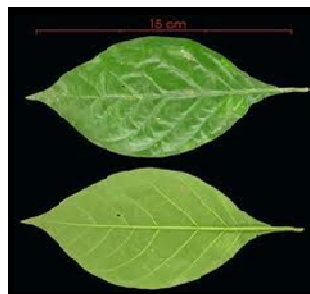


fig.1-*Catunaregam spinosa* tree, fig.2- Flowers, fig.3-fruit, fig.4-fruit inside, fig.5-leaves

TS of fruit

The fruit is circular in cross sectioned view with four shallow, less prominent longitudinal grooves. The cleared fruit showing epidermis, embryo, pericarp, placental tissue, vascular strand and calcium oxalate crystals.

Fig.6.T.S of placenta and pericarp

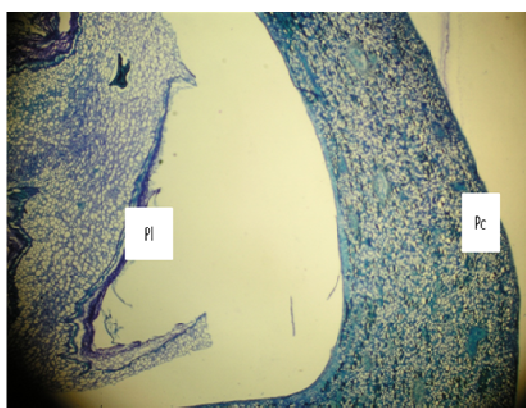
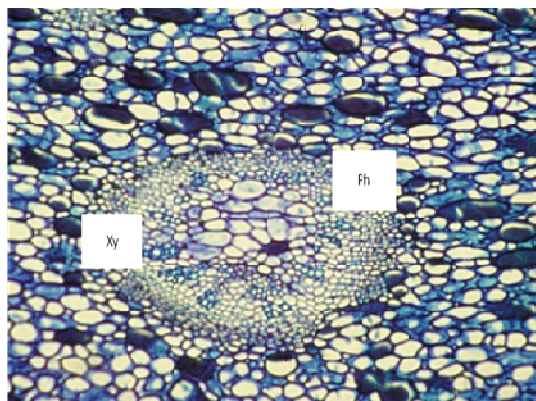


Fig.7.A sector of the Pericarp



(Pl- Placental tissue Pc-Pericarp Xy- Xylem Ph- Phloem)

Fig -8 T.S of vascular strands of pericarp

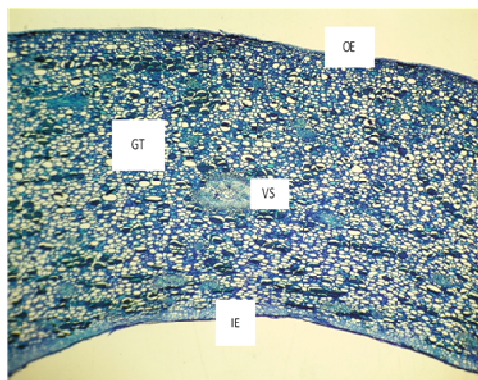


Fig.9 T.S of Embryo in the placentas

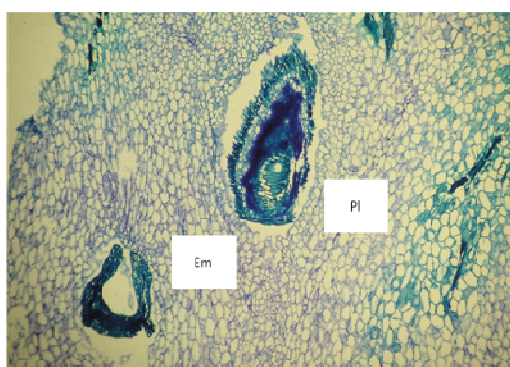


Fig.10.Calcium oxalate crystal in the placental tissue

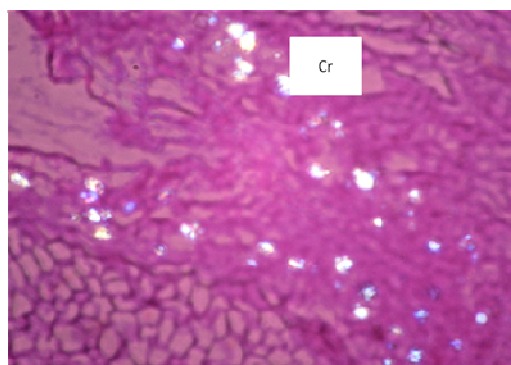
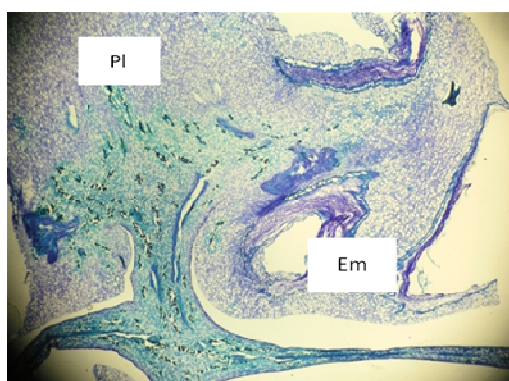


Fig.11.Placental tissue sharing the embryo



(EM- Embryo Cr-Calcium oxalate crystals GT-Ground tissue
IE-Inner epidermis OE - Outer epidermis VS-Vascular strand)

Table.1 Data showing the Physico – Chemical Standards of fruit powder of *Catunaregam spinosa*

S. No	Total Ash %w/w	Acid In Soluble Ash %w/w	Water soluble Ash %w/w	Sulphated Ash %w/w	Loss on Drying %w/w	Alcohol Soluble Extractive % w/w	Water Soluble Extractive % w/w	Crude Fibre Content % w/w
1.	10.3	1.5	2.4	4.5	1.95	11.2	0.8	10.6
2.	10.4	1.8	1.9	4.3	1.88	12.60	0.81	10.8
3.	10.2	2.0	2.0	4.9	1.96	12.24	0.9	11.5
4.	10.4	1.7	2.2	5.0	1.95	14.36	0.78	11.8
5.	10.3	1.9	2.3	4.6	1.86	15.66	0.82	11.6
Minimum	10.2	1.5	1.9	4.3	1.92	11.52	0.78	10.6
Average	10.3	1.7	2.2	4.6	1.86	13.28	0.82	11.26
Maximum	10.4	2.0	2.4	5.0	1.96	15.60	0.9	11.8

Table 2. Fluorescence Analysis of Extracts and Drug powder of *Catunaregam spinosa*

Reagents	Petroleum ether extract		Chloroform extract		Acetone extract		Methanol extract		Aqueous extract		Drug powder	
	DL	UVL	DL	UVL	DL	UVL	DL	UVL	DL	UVL	DL	UVL
Extract as such	Green	Green	Yellow	Yellow	Greenish yellow	Light green	Brownish yellow	Brownish yellow	Dark brown	Dark green	-	-
1 N Sodium Hydroxide (aqueous)	Yellowish green	Dark green	Yellowish brown	Dark green	Yellowish brown	Green	Yellow	Yellow	Yellowish brown	Greenish yellow	Pale green	Yellowish green
1 N Sodium Hydroxide (alcohol)	Yellowish green	Yellowish brown	Yellow	Yellow colour	Brown	Greenish black	Yellowish brown	Pale green	Brownish yellow	Greenish brown	Green	Fluorescent green
1 N Hydrochloric Acid	Pale green	Green	Brown	Green colour	Yellowish black	Brownish green	Yellowish brown	Brownish yellow	Pale yellow	Pale yellow	Brown	Light green
50% Nitric Acid	Dark green	Brownish yellow	Greenish yellow	Yellow colour	Yellowish black	Yellowish black	Brownish black	Brownish green	Pale yellow	Greenish yellow	Green	Fluorescent green
50% Sulphuric Acid	Pale yellow	Yellow	Green	Yellowish green	Brownish green	Pale yellow	Pale brown	Yellowish green	Whitish yellow	Pale green	Green	Fluorescent green
Methanol	Brownish green	Brown	Light green	Green	Greenish yellow	Yellowish green	Dark brown	Brownish green	Pale yellow	Yellow	Green	Fluorescent green
Ammonia	Pale brown	Greenish brown	Greenish yellow	Dark green	Green	Green	Greenish yellow	Dark green	Brownish yellow	Dark green	Green	Yellowish green
Iodine	Reddish brown	Yellowish brown	Brownish yellow	Yellowish brown	Reddish yellow	Greenish yellow	Yellowish brown	Pale green	Black	Brownish green	Brown	Light green
Ferric Chloride	Dark brown	Dark green	Greenish brown	Yellow	Black colour	Black	Black	Black	Dark brown	Blackish green	Green	Light green

DL-Day Light, UVL-UV Light

Quantitative standards of powdered fruit of *Catunaregam spinosa*:

Ash values including total ash, acid insoluble ash and water soluble ash of fruit powder were done as per Indian Pharmacopoeia. The results are shown in Table 1. Successive extractive value with different solvents with fruit powder were done. The colour, consistency and percentage of extractive values shown in Table 3. The fluorescence analysis of powdered drug as well as its extractives in daylight and UV light were examined. The observations are given in Table 4. The presence or absence of different phytoconstituents, viz. alkaloids, carbohydrate, glycosides, flavonoids, terpenoids, saponins and phenolic compounds were detected by prescribed methods and results are given in Table 5.

Table.3 Successive Solvent Extraction of *Catunaregam spinosa*

S.NO	Extracts	Colour and Consistency	Percentage yield of extracts of <i>Catunaregam spinosa</i> w/w
1.	Petroleum ether (60 – 80%)	Green & Viscous mass	1.48
2.	Chloroform	Green with sticky mass	1.05
3.	Acetone	Greenish yellow colour & Semisolid in nature	1.22
4.	Ethanol	Yellow colour & Semisolid in nature	8.86
5.	Aqueous	Brown colour powder	6.35

Table.4 Data showing the preliminary phytochemical screening of *Catunaregam spinosa*

Phytoconstituents	Petroleum ether extract	Chloroform extract	Acetone extract	Methanol extract	Aqueous extract
Alkaloids	(-)	(+)	(-)	(+)	(+)
Carbohydrates	(-)	(+)	(+)	(+)	(+)
Glycosides	(-)	(+)	(+)	(+)	(+)
Flavonoids	(-)	(-)	(-)	(+)	(+)
Phytosterols	(+)	(-)	(-)	(+)	(-)
Fixed oils and Fats	(-)	(+)	(-)	(+)	(+)
Saponins	(-)	(-)	(-)	(+)	(+)
Phenolic compounds and Tannins	(-)	(-)	(+)	(+)	(+)
Lignins	(+)	(-)	(+)	(+)	(+)
Proteins and free Amino acid	(-)	(-)	(-)	(+)	(+)
Gums and Mucilage	(-)	(-)	(+)	(-)	(+)

(+) Positive, (-) Negative

CONCLUSION

The plant *Catunaregam spinosa* is a plant used as a traditional medicine and can be well exploited for various pharmacognostical studies. Amongst the chemical classes present in this plant species, alkaloid, saponin, flavanoid, glycoside, carbohydrate and phenolic compounds stand as a class of major importance in the development of new drugs. The present investigation has stated important standardisation parameters of macro and microscopical characters, ash and extractive values, phytochemical screening would be useful in authenticating *Catunaregam spinosa* Linn.

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