

## Pharmacological Study of a Steroid-sitosterylglucoside-6'-heptadecanoate Isolated from Capparis horrida

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### ABSTRACT

Pharmacological properties of the species Capparis horrida has been known among the tribal population of Odisha since long. The aqueous extract of this plant material is related to anti-inflammatory and analgesic activities. It has also been known for its medicinal properties as anthelmintic, diuretic and aperient. The ethanolic extract of this plant species was subjected to chromatographic techniques using silica gel and benzene: petroleum benzene (1:9) to isolate a new steroid-sitosterylglucoside-6'-heptadecanoate as the major compound. The TLC was studied using ethyl acetate: benzene (1:9) the  $R_f$  value of the compound is 0.63. The structure of the compound was established from the IR,  $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$  and mass spectral data. The elemental analysis of the compound supported these spectral data. The bioassay of the isolated material was studied using frog rectus abdominis muscle. The acetylcholine liberated from the nerve ending of the muscle caused a depolarization of the muscle fiber. From the kymograph it is observed that the compound caused muscle contraction in frogs. The antimicrobial activity was studied by two-fold serial dilution method. The anti-bacterial activity of the compound was compared with Gentamycin which is a known drug having this activity. The compound showed bacterial growth-inhibiting property.

**Keywords:** Capparis horrida, Cappariaceae, stem, steroid, bioassay, acetylcholine, kymograph.

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### INTRODUCTION

Root bark of Capparis horrida (Cappariaceae) is known in folk medicine to possess aperient, tonic, diuretic, expectorant, anthelmintic, emmenagogue and analgesic effect [1, 2]. Capers are said to reduce flatulence and are anti-rheumatic. Also it is reported that the juice of the fresh plant kills the worms in the ear [2] and the dried leaves steeped in vinegar are used for treatment of ulcers [3]. Previous studies on this plant led to isolation of stachydrin [4], plicatol [5], flavonoids [6-9] and glucosinolates [10].

We have carried out the investigation in the tribal areas of Ganjam and Gajapati districts of Odisha in India where the tribal healers successfully use the stem and root part of Capparis horrida for the orthopedic and rheumatic purposes. The present study deals with the isolation, characterization and biochemical study of active ingredients,

which are responsible for curing the diseases with a minimum period of time and with high degree of success.



### MATERIALS AND METHODS

The stem and root parts of the plant Capparis horrida were collected locally from hilly parts of southern Odisha, India in the month of June. The plant material was identified by the botanist of our institute

and the specimen (file No. XII) is preserved in the herbarium.

Kymograph, student organ bath, aeration rate aerator, aeration tube holder, frontal bioassay of writing lever, lever holder screw clip, haemostatic forceps, needle, scissors, forceps, thread plasticine, gram weight were used.

### Experimental design

The dried ethyl alcohol extract was treated with distilled water. A greenish colored mass was obtained after filtration by using Whatman-42 filter paper. Again the residue was thoroughly dissolved in ethyl alcohol. The purity was checked by using TLC in a solvent system of benzene: ethyl acetate (2:1) ratio. The residue was chromatographed over silica gel, eluting with petroleum benzene and benzene (9:1). The isolated compound was subjected to biochemical studies to observe its activities on broken muscle tissues of frog and its effect on bacteria culture.

The frog was pith by passing a pithing needle through the occipitoatlantic junction between the brain and the spinal cord. The stretching out of the limbs indicates that the pithing is proper. The frog was placed in a dissection tray. The skin of abdomen was picked up with the help of forceps and made proper incision to expose the rectus abdominis muscle. The rectus abdominis muscle was cut and freed from the anterior abdominal vein. The muscle was transferred to petri-dish containing frog Ringer solution. The muscle was tied with short thread to the hook of the aeration tube and was placed in the inner organ bath containing frog Ringer solution. A long thread was tied to a frontal writing lever. The load on the lever was maintained 1 gram. The magnification was between 5-7 times. The rectus muscle was stabilized for 55 minutes period. During the stabilization period the frog Ringer solution was replaced in the inner organ bath at an interval of 5 minutes. After stabilization for a period of 55 minutes kymograph was switched on and the normal tracing was recorded for a period of 30 seconds. At the end of 30 seconds, acetylcholine (0.1 ml) solution was injected into the inner organ bath and the response was recorded for a period of 90 seconds (Material Contact Time). At the end of 90 seconds the

Kymograph was switched off and 3-4 washings of rectus muscle with frog Ringer solution was given. Thereafter, 0.1 ml of acetylcholine solution was injected into the inner organ bath once again and the response was recorded for 90 seconds. 0.1 ml of the test sample was injected and the response was recorded for 90 seconds comparing with that of the response produced by submaximal dose of the standard acetylcholine solution.

Bacteria culture was done on nutrient broth (Himedia) at 37° C for 15 hours. The anti bacterial activity of the extract was determined against *Escherichia Coli*, *Staphylococcus aureus* and *Bacillus cereus*. The anti bacterial was studied by two fold dilution method minimum inhibitory concentration (MIC) was recorded at the lowest concentration in which no bacterial growth was observed (Table1).

## RESULTS AND DISCUSSION

### a. Analytical methods

Column chromatography was carried out using silica gel 60-120 mesh (Ranbaxy) and TLC was carried out using silica gel G on a Bruker DRX 300 spectrometer, with TMS as an internal standard.

The stem part of *Capparis horrida* afforded a new steroid identified as -sitosteryl-glucoside-6'-heptadecanoate.

The <sup>1</sup>H NMR of the new compound (CDCl<sub>3</sub>, 300 MHz); 0.65 (3H, s, C<sub>18</sub>-H), 0.08-0.98 (overlapped doublet, CH<sub>3</sub>), 1.25 (huge broad, fatty acid chain), 1.47-2.00 (complex CH<sub>2</sub> and CH), 2.34 (2H, t, J=7.5Hz, CH<sub>2</sub>COO), 3.36 (1H, m, C<sub>3</sub>-H), 3.51-4.37 (several multiplets, sugar moiety), 5.34 (1H, m, C<sub>6</sub>-H). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 125MHz); 11.9, 12.0, 14.1, 18.8, 19.0, 19.8, 21.1, 22.7, 23.1, 24.3, 25.0, 26.2, 28.3, 29.2, 29.4, 29.6, 29.7-29.8, 31.88, 31.95, 34.0, 34.3, 36.2, 36.7, 37.3, 38.9, 42.3, 45.8, 50.2, 56.2, 56.8, 63.9, 70.6, 73.4, 73.7, 79.8, 101.3, 122.0, 140.4, 174.1, FAB-MS; m/z 88 [C<sub>5</sub>H<sub>12</sub>O], 270 [C<sub>17</sub>H<sub>34</sub>O<sub>2</sub>], 414 [C<sub>29</sub>H<sub>50</sub>O].

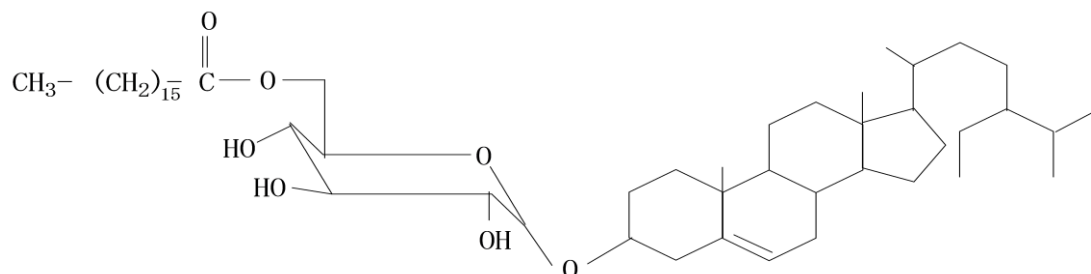
The following structure has been assigned to the compound from the IR, H-NMR, <sup>13</sup>C-NMR and mass spectral analysis data.

### b. Bioassay

The bioassay of this isolated material from the plant *Capparis horrida* was studied by comparative method using frog rectus abdominis muscle which is a voluntary muscle. At the neuromuscular junction, a

nerve impulse liberates acetylcholine from the nerve ending into the cleft between muscle and nerve fiber. This acetylcholine causes a depolarization of the muscle fiber where a depolarization of the muscle fiber occurs, which in turn sets of a muscle action potential and concentration of muscle fiber.

From the Kymograph it appeared that the contraction of muscle tissue by the application of the compound indicated the effect of the active ingredient isolated from the plant species *Capparis horrida* from the traced curves by comparative method.



**Fig.-sitosterylglucoside-6'-heptadecanoate**

**Table 1: Anti Bacterial activity of -sitosterylglucoside-6'-heptadecanoate**

Bacteria	Minimum inhibitory concentration (µg/ml) Compound	Gentamycin
Gram (+)	9.5	6.24
Bacillus cereus		
Staphylococcus aureus	10.3	24.7
Gram(-)	2.10	6.23
Escherichia Coli		

## CONCLUSION

From the present study it is found that the isolated compound could be a contributing factor leading to the healing of muscle tissues. It also has anti bacterial activity. This will be helpful in scientifically correlating the practical observation made by traditional healer regarding its successful application on wounds and broken muscle tissues.

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