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PHYTOCHEMICAL SCREENING ON THE STEM BARK OF *ANTHOCEPHALUS CADAMBA (ROX B.)* MIQ.

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ABSTRACT

The present work deals with development and standardization of phytochemical screening for quantification of methanolic extract of medicinal plant stem bark of *Anthocephalus cadamba (Roxb.)Miq.* The scientific parameter is necessary to identify the exact plant material and to find its quality and purity. The present study deals with various, physical evaluation and preliminary phytochemical screening of various successive extracts such as qualitative chemical analysis and different spectroscopic study were carried out and the parameters were reported. HPTLC of extract shows the seven different peaks confirming that the seven compounds present in methanolic extract of *Anthocephalus cadamba*. The alkaloidal content of stem bark of the title plant has been determined. These studies indicated the possible information for correct identification and standardization of this plant material.

INTRODUCTION: *Anthocephalus cadamba (Roxb.)* Miq. family *(*Rubiaceae) is a deciduous tree of occasionally buttressed up to 37.5 m in height and 2.4 m in girth; with a clear bale of 9m and horizontal branches, found all over India and also cultivated. Stem Bark grey, fissured, leaves coriaceous, broadly ovate, elliptic-oblong, 7.5-18.0 cm x 4.5-16.0 cm; flower heads globose, yellow, solitary; terninal, 3.7 cm in diameter consisting of small ; yellow or orange-colored, scented flowers; fruit a fleshy, orange, globose pseudocorp of compressed angular capsules with persistent calyx; seeds small, muriculate.[1] Vernacular Names: - Hindi : Kadamb , English: Cadamba, Malayalam: Karamu, Kannada: Kaduavalatige Telugu: Rudrakshamba,.

The various part of the tree is widely used in Ayurveda, Siddha and Unani system of medicine. *Anthocephalus cadamba stem bark* is reported to possess tonic, astringent febrifugal and anti-diuretic properties and is given in cough. The juice of the bark forms constituents of a compound to treat in flammation of the eye. The dried bark has been reported to contain steroids alkaloids; fats and reducing sugar and also used for Improvement of Semen Quality. The major constituents of bark are triterpenes, triterpernoids, glycosides, indole alkaloids.[5]

Kumar V., *et al* (2008) studied the lipid lowering activity of *Anthocephalus indicus* (family Rubiaceae; Hindi name Kadamba) root extract in triton WR-1339 induced hyperlipidemia in rats. Kapil A., *et al* (2002) screened the effect of chlorogenic acid (CGA) isolated from *Anthocephalus cadamba* for hepatoprotective activity by *in vitro* and *in vivo* assay methods using carbon tetrachloride (CCl_4) as a model of liver injury . Umachighi S.P., *et al* (2007) had reported antibacterial, wound healing and antioxidant activity of bark of *Anthocephalus cadamba*. The objective of this work is too carried out phytochemical screening to isolate active constituents of methanolic extract of *A. cadamba.*

MATERIAL AND METHOD:

Plant Material: The stem bark of *Anthocephalus cadamba (Roxb.)Miq.* was collected from Orai, Distt- Jalaun (U.P.). The plant materials was identified and Authenticated by Dr. Gaurav Nigam, Department of Botany, Institute of basic Science, B. U. Jhansi (U.P.), India. *Ref. No*- B.U./Bot./375/24-01-09.

Preparation of Extract: The stem bark of *Anthocephalus cadamba (Roxb.)Miq.* shaded dried, and then these are made into coarsely powdered form using dry grinder. The powdered bark of the plant (180gm.) was packed in soxhlet apparatus and continuously extracted with petroleum ether (40-60^{0}C) till complete extraction, after completion of extraction the solvent was removed by distillation and then concentrated extract obtained was dried under reduced pressure using rotatory evaporator at temperature not exceeding 40^{0}C and then give moderate heating on water bath.

A yellowish extract approximate 1 gm. was obtained. From the drug petroleum ether was removed and the defatted drug was extracted with methanol (95%) till complete extraction, after completion of extraction the solvent was removed by distillation and then concentrated extract obtained dried under reduced pressure at temperature not exceeding 40^{0}C and then give moderate heating on water bath. The methanolic extract

obtained was dark yellow in color, weighed about 42.8 gm. The methanolic extract was kept in Petridis and it was stored in desiccator at cool place [2].

Experimental Method:
Phytochemical Tests:

TABLE 1: PHYTOCHEMICAL TEST METHANOLIC EXTRACT OF *ANTHOCEPHALUS CADAMBA* [2]

TESTS	METHANOLIC EXTRACT
Alkaloids	+
Carbohydrates	+
Proteins	-
Flavonoids	+
Glycosides	+

Thin Layer Chromatography:

Preparation of Plates: Silica gel G was used as the adsorbent. Slurry of it was prepared with distilled water in a glass pestle mortar. The slurry was poured on the clean and dry glass plates and spread on the plate as a uniform coating using a glass rod. These plates were then placed on a leveled surface in the horizontal position and allowed to air dry for 20-25 minutes.

Activation of Plates: When the plates were dried they were placed in an oven, maintained at 110^0 C for 30 minutes. The prepared plates were stored in a closed desiccated cabinet and removed only when required for use.

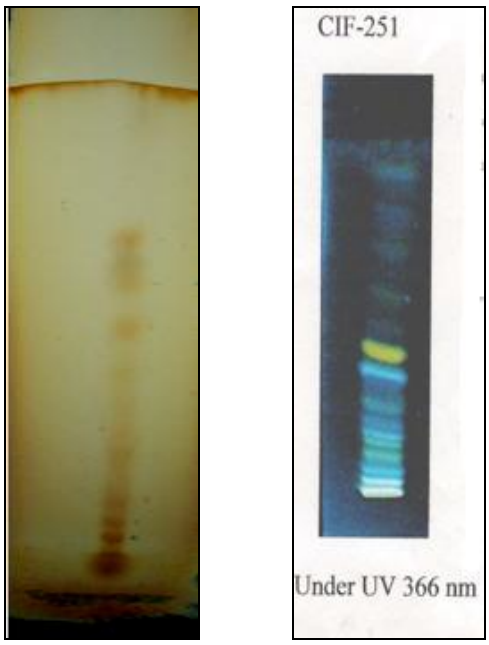
Preparation of Samples: About 100 mg of test material was dissolved in 10 ml of the respective solvent and was used for the TLC studies.

Application of Spots: The spots were applied on the activated plate at a distance of 2 cm from one end of the plate and 3 cm from each other with the help of a fine capillary tube or diameter less than 1 mm. The solvent was removed from spot by air-drying. The position of the origin was marked.

Development of Chromatograms: Chromatograms were developed by one way ascending TLC. The plate carrying spots was placed squarely in the developing chamber and the lid was replaced as quickly as possible to minimize disturbance of the solvent saturated atmosphere. The developing solvent was allowed to travel up the plate until it reached the desired level (10 to 15 cm). The plate was then removed from the chamber; solvent front was marked and dried in air at room temperature [3].

Detection of Spots: The number and position of the various constituents present in the mixtures was determined by spraying the plate with the 1% vanaline in sulphuric acid and the plate was heated at 110 ^{0}C for 10 minutes and the spots were marked. R_f value was calculated for well [4, 5, 6].

Observation: The methanolic extract of cadamba (rox b) showed the best result in the solvent system ethyl acetate: methanol: water: formic acid (16:0.2:1.6:0.7) and gave 7 spots. Their R_f values were 0.06, 0.10, 0.14, 0.40, 0.50, 0.60, 0.70.



(A) (B)

FIG. 1: (A) TLC CHROMATOGRAM OF METHANOLIC EXTRACT OF *ANTHOCEPHALUS CADAMB;* (B) HPTLC CHROMATOGRAM OF METHANOLIC EXTRACT OF *ANTHOCEPHALUS CADAMBA* ON THE UNDER 366 nm.

High Performance Thin Layer Chromatography (HPTLC): High performance thin layer chromatography was carried out by National Botanical Research Institute, Lucknow (U.P.). It also gives seven numbers of peaks. The analysis results are shown in fig. 2 and table 2:

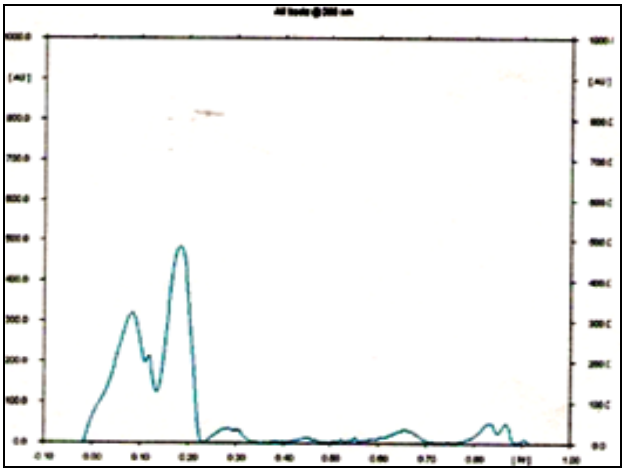


FIG. 2: HPTLC PEAKS OF METHANOLIC EXTRACT OF *ANTHOCEPHALUS CADAMBA*

TABLE 2: HPTLC PEAKS OF METHANOLIC EXTRACT OF *ANTHOCEPHALUS CADAMBA* WITH R_F VALUES

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area%	Assigned Substance
1	1	0.01 Rf	92.0 AU	0.08 Rf	320.3 AU	32.57%	0.14 Rf	124.5 AU	15544.6 AU	42.80%	unknown *
1	2	0.14 Rf	126.2 AU	0.18 Rf	484.1 AU	49.22%	0.23 Rf	0.5 AU	16108.8 AU	44.36%	unknown *
1	3	0.24 Rf	0.2 AU	0.29 Rf	35.2 AU	3.58%	0.34 Rf	2.0 AU	1287.1 AU	3.54%	unknown *
1	4	0.42 Rf	5.1 AU	0.45 Rf	13.6 AU	1.38%	0.48 Rf	1.7 AU	300.9 AU	0.83%	unknown *
1	5	0.61 Rf	13.6 AU	0.65 Rf	33.6 AU	3.42%	0.71 Rf	1.9 AU	1224.0 AU	3.37%	unknown *
1	6	0.77 Rf	2.5 AU	0.83 Rf	48.7 AU	4.95%	0.85 Rf	25.7 AU	1225.0 AU	3.37%	unknown *
1	7	0.85 Rf	26.2 AU	0.87 Rf	48.0 AU	4.88%	0.88 Rf	3.4 AU	626.8 AU	1.73%	unknown *

Sample Preparation- 10mg/ml
Application-Linomat 5 Applicator (Camag)
Volume applied-10 µl
Solvent System- Ethyl acetate: Methanol: Water: Formic acid (16:0.2:1.6:0.7)
TLC plate Development-Presaturated Camag Twin Trough Chamber

Characterization of Isolated Compound: The compound which is isolated in column chromatography is characterized by the analytical techniques such as Infrared spectroscopy, NMR spectroscopy and Mass spectroscopy.

Infrared Spectroscopy: Infrared spectroscopy is generally sensitive to the presence of functional groups in the samples. The most powerful aspects of Infrared spectroscopy are that it allows identification of unknown compound. IR spectroscopy of compound (G-9) was performed in CDRI, Lucknow. Spectra of compound have shown in figure 3 the interpretation that can be made from spectra has shown in table 3.

TABLE 3: INTERPRETATION OF IR SPECTROSCOPY

WAVE NUMBER (cm-1)	FUNCTIONAL GROUP
3466.1	O - H stretching in alcohol group
2987.0	C - H stretching in alkane
1745.5	Ketone stretching 5 membered ring
1375.2	O - H Bending in alcohol and phenol
1243.5	C - NO unsaturated nitrogen

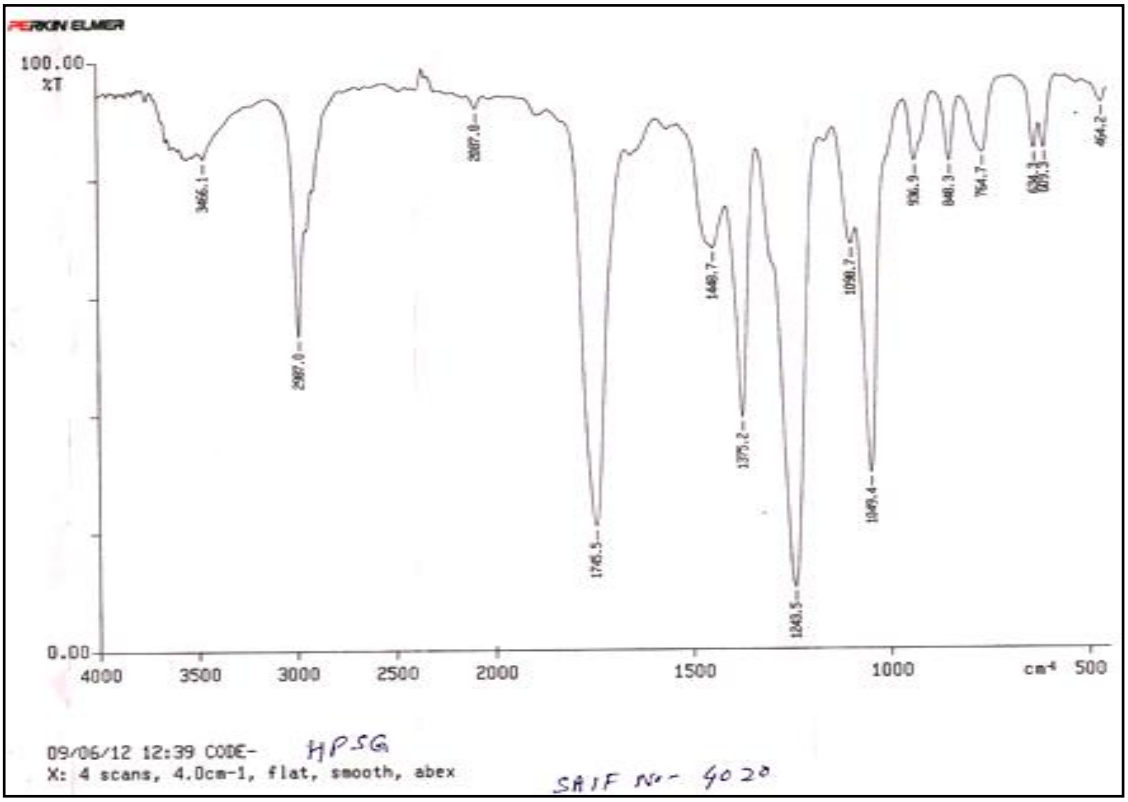


FIG. 3: IR SPECTRA OF COMPOUND G-9

Mass Spectroscopy: The mass spectroscopy of compound (G-9) was performed at C.D.R.I. Lucknow, The mass spectra is used to determine the possible fragmentation in the compound. The mass spectra of compound have shown in Fig. 5.4 the spectra exhibited various peeks suggesting fragmentation pattern. Fragmentation data of isolated compound (G-9): 95, I07, 123, 136 149,154 and is given in table 4 and figure 4.

TABLE 4: INTERPRETATION OF MASS SPECTROSCOPY

m/z	RELATIVE INTENSITY
369	15
337	25
326	15
261	100
149	70
154	98

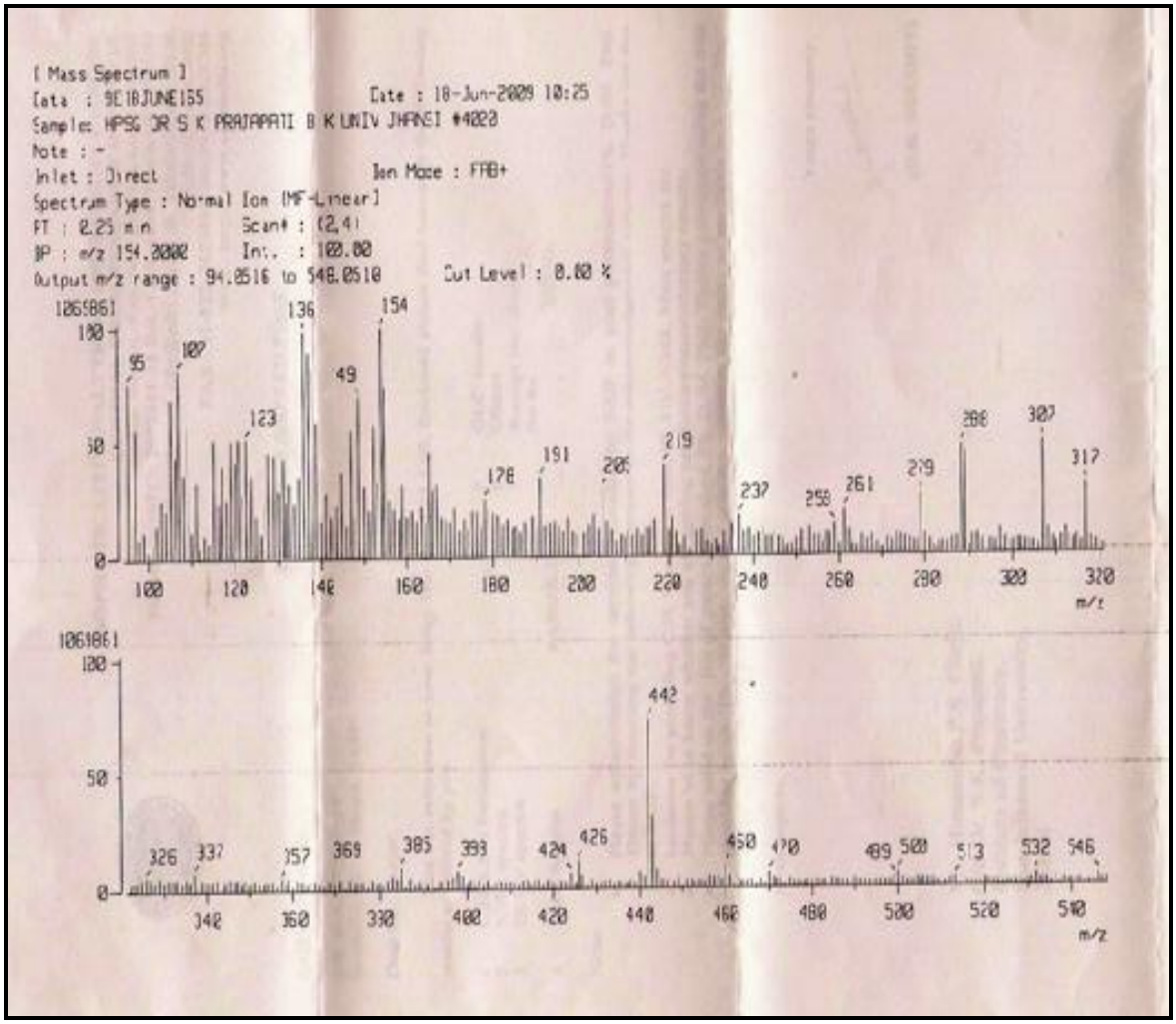


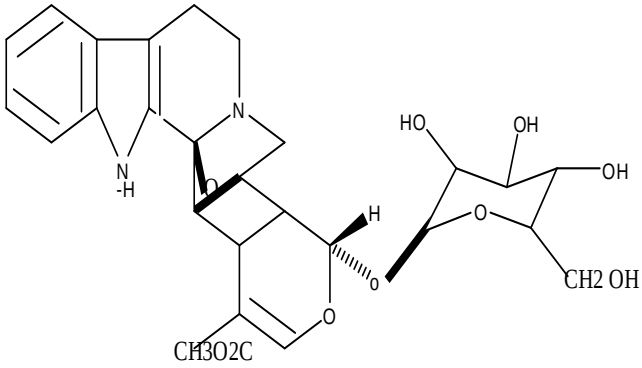
FIG. 4: MASS SPECTRA OF ISOLATED COMPOUND G-9

NMR Spectroscopy: The NMR spectroscopy of compound (G-9) was performed at C.D.R.I. Lucknow, The mass spectra is used to determine the possible Proton in the compound. The NMR spectra of compound have shown in table 5 and figure 5.

TABLE 5: INTERPRETATION OF NMR SPECTROSCOPY

δ VALUE (ppm)	ASSIGNMENT
1.3-5.5	Hydroxyl R-OH
2.0-2.2	Ester H-C-COOR
3.0-3.5	Alcohol
4.2	Phenolic
5.0	Enolic

Analytical Result of Isolated Compound G-9: In Cadambine, functional group OH, CH, Ketone stretching 5 membered ring, hydroxy R-OH, alcohol, H-C-COOR present. According to above study, the isolated compound is cadambine, color has pale yellow and R_f values of isolated compound 0.86.



Chemical name: Cadambine
Melting Point: 221 °C
Color: colorless crystalline which become pale yellow

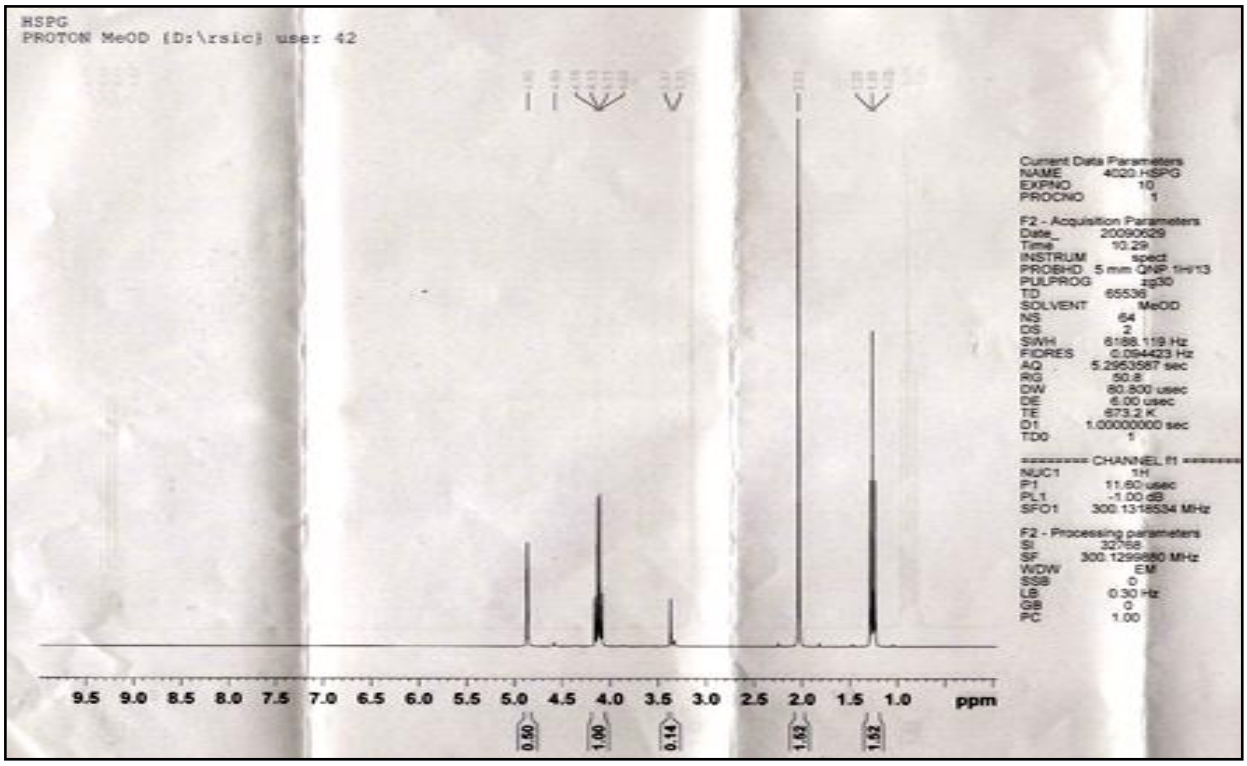


FIG. 5: NMR SPECTRA OF ISOLATED COMPOUND G-9

RESULT AND DISCUSSION: Phytochemical evaluation of methanolic extract of *Anthocephalus cadamba (Roxb.)Miq.* showed the presence of flavonoids, alkaloids, carbohydrate, proteins, and glycoside compounds. Best result of Thin Layer Chromatography in the solvent system: Ethyl acetate: methanol: water: formic acid (16: 0.2: 1.6: 0.7) was found in which 7 spots were present with R_f values 0.06, 0.10, 0.14, 0.40, 0.50, 0.60, 0.70 same solvent system. By analytical study, it was found that Cadambine compound get isolated from methanolic extract of *Anthocephalus cadamba*.

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