PHYTOCHEMICAL& IN VITRO PHARMACOLOGICAL SCREENING OF PLANT BENTECA RHEEDI EXTRACT

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ABSTRACT

This study's goal was to investigate possible Anti ulcer and Coagulant properties of various extracts of *Benteca rheedi*, a plant traditionally used in Ayurveda and Unani medicine. Preliminary phytochemical analysis was performed to determine the composition of the extracts. In vitro assays were carried out to evaluate the potential anti-ulcer activity by measuring Acid Neutralizing capacity (ANC), as well as Coagulant activity by using Prothrombin Time Test (PTT) ,the findings demonstrated that the Methanolic extract at 1000mg concentration showed a significant Anti ulcer activity using Acid neutralizing capacity at 16.6 (ANC per gram of extract), and The Methanolic extract at 200mg concentration showed a significant coagulant activity at 85 sec. The presence of phytochemical compounds such as alkaloids, tannins, saponins, steroids, flavonoids ,terpenoids, and phenolic compounds in the extracts of *Benteca rheedi* may explain the observed pharmacological activities of the plant. In conclusion, these extracts have the potential to be developed as natural anti-ulcer and coagulant agents.

Keywords: *Benteca rheedi*, methanolic extract, phyto chemical screening, anti-ulcer, coagulant activity.

MEDICINAL PLANTS USED IN CHEMISTRY :

Between 350,000 and almost half a million species of vascular plants, or 10% of all plants, are thought to be utilized as medicines. Plants have been utilized in medicine since ancient times and are being used now. Trial and error were initially employed to identify helpful plants with good effects, whether it was to treat ailments or simply to feel better. Traditional medicine is a term that refers to the progressive refinement of the usage of these herbs through many generations. Traditional medicine is described as "the totality of knowledge, skills, and practices based on indigenous theories, beliefs, and experiences" in the official definition. Whether explicable or not, several cultures employ these practices to maintain health as well as to prevent, diagnose, treat, or ameliorate various physical and mental disorders.[1-6]

All civilizations have produced this type of medicine based on the plants in their own environments, it is a truth. Even some authors have asserted that the history of pharmacy and medicine may be traced back to this communicated information. Numerous higher plants are still being grown today for their medicinal and pharmaceutical benefits. Due to various plants' therapeutic qualities, medical medications with these advantages have been created. The medicinal benefits of several plants, their impact on the human body, and their methods of use were recognized up to the 18th century, although the that are For instance, until the 18th century, the Persian physician and scholar Avicenna (Ibn Sina)'s Canon of Medicine was in use.[6-12]

PLANT INTRODUCTION:

Benteca rheedi is a genus of flowering plants in the family Rubiaceae. It has about 30 species. All are native to the Old World. *Bentica rheedi* possesses a soft, limited-use wood that is mostly used for boxes.[13,14] The type In an addition to William Roxburgh's Flora Indica that was published by Carey and Wallich after Roxburgh's passing, Nathaniel Wallich gave the plant its name in 1824. species for Benteca is Benteca rheedi (synonym: Hymenodictyon excelsum).[15,16]. Molecularphylogenetic studies haveshownthat Bentica rheedi is paraphyletic over the Madagascan genus Paracorynanthe .Large deciduous glands known as colleters are present on the stipules of Bentica rheedi and Paracorynanthe. The corolla tube is small at the base and gets bigger as it gets closer to the tip. It looks like a woody capsule.[17-20] A common Thai medicinal plant, *Bentica rheedi*. (syn. H. Excelsum), is found in mixed forests and rainforests in northern, central, and southern Thailand. In Thai, it is referred to as "U Lok" and "Som K This plant is a deciduous tree that reaches heights of 9 to 12 meters. While the bark of this plant is mostly employed as febrifuge and astringent, the leaves have historically been used to treat inflammation, sore throat, tonsillitis, sinusitis, and ulcers Previous studies on this genus led to the isolation of coumarins iridoids, anthraquinones, triglyceride, steroids and acetylenic fatty acids whereas chemical investigation of the bark of this plant provided β-sitosterol, stigmasterol and coumarins, hymexelsin, aesculin and scopoletin. A potential AChE inhibitor that restores neurotransmitter deficit in Alzheimer's disease is coumarins The MeOH extract of *Benteca rheedi* bark demonstrated little inhibition of AChE at 500 mg by TLC bioautography test in a screening for AChE inhibitors A member of the Rubiaceae family *Benteca rheedi* is widespread over most of India and is a native of tropical Asia and Africa.

Benteca rheedi, which grows wild from Bago Yoma to the hill areas of upper Myanmar, is utilized in indigenous medicine in Myanmar. It generates alkaloids and scopoletin, which are used as febrifuges, as a quinine alternative in traditional medicine, as probable regulators of tumor promotion, and as a remedy for sore throat and appetite.[21-24] The system of traditional medicine in Myanmar has existed for many hundred years. Traditional and herbal treatments from Myanmar are becoming more and more well-liked and acknowledged. Ku-than is a less well-known plant constituent that is frequently utilized in Myanmar, it has been noted. by those who practice traditional medicine. Although Ku-than was not a widely utilized substance like Cinchona, which was used to cure malaria, its usage as a substitute for or comparable to Cinchona should prompt further research into the plant's ingredients. As a result, the current study's goal is to learn more about and identify the chemical components of ku-than barks.[25-27]

MATERIALS AND METHODS

Materials

FIG: 1 Benteca rheedi

Taxonomical study of Bentica rheedi

- Kingdom: Plantae
- Phylum: Tracheophyta
- Class : Mangoliopsida
- Order: Gentianales

- Family: Rubiaceae
- Genus: Benteca
- Species: rheedei

Plant collection and authentication :

The fresh leaves and dried bark of *Bentica rheedi* were collected during the months of December-January from the Horsley Hills. The plant material was taxonomically identified and authenticated by Dr. Madhava Cheety, Department of Botany, Sri Venkateswara University, Tirupati. The flower voucher number-0417. The fresh leaves and dried bark were collected and kept shaded dry for 15 days. The dried bark and leaves were taken and milled into coarse powder by a mechanical grinder and stored in a airtight container

MATERIALS / CHEMICALS USED

The chemicals used in the experiment they are methanol, NAOH, and sulphuric acid, by SDFCL Laboratories from Mumbai.

Extraction techniques of Medicinal plants

The process of separating the medicinally active components of plant or animal tissues from the inert or inactive components is known as extraction in the pharmaceutical business. Extraction techniques of Medicinal plants ones using certain solvents. in standard extraction procedures. The somewhat impure liquids, semisolids, or powders produced in this way by plants are only fit for external or oral consumption. Decoctions, infusions, fluid extracts, tinctures, pilular (semisolid) extracts, and powdered extracts are some of these preparation types. These remedies are known as "galenicals," after the Greek physician Galen, who lived in the second century. Standardized extraction techniques are used to get the therapeutically needed amount of crude medicines and to remove the inert material by using a selective solvent that is as menstruum. The resulting extract can be processed further to be included in any dosage form, such as tablets or capsules, or it can be fractionated to isolate specific chemical entities, such as the modern drugs ajmalicine, hyoscine, and vincristine. The resultant extract can also be made into tinctures and fluid extracts that are prepared for use as pharmaceuticals. Thus, uniformity of extraction processes has a considerable impact on the herbal drug's ultimate quality.

SOXHLET (HOT CONTINUOUS EXTRACTION) This technique involves placing the coarse medication that has been coarsely pulverized into a porous bag or "thimble" made of sturdy filter paper and placing it inside the Soxhlet device.. The extracting solvent in flask A is heated, and its vapours condense in condenser. The crude medication is extracted by contact as the condensed extractant drops into the thimble holding it. The liquid within the chamber siphons into the flask when the level reaches the siphon tube's top

Fig:2 soxhlet apparatus

PREPARATION OF EXTRACTION

The plant material was dried under shade at room temperature for about 10 days.the dried plant samples were powdered by mechanical grinder and sieved to give particle size 50 to 150 mm. the powder was stored in polythene bags at room temperature before extraction powder (100g) was filled in the thimble and extracted successively with methanol in a soxhlet extractor. 16 hours of extraction were spent until the solvents' colors returned to normal at the end of the siphoning process. Using rotary evaporator equipment, solvents were evaporated at low pressure. This operation is carried out continuously until an evaporated drop of solvent from the siphon tube leaves no residue. Comparing this procedure to others previously

mentioned, the benefit is that significantly less solvent is needed to extract huge quantities of medication. Time, energy, and subsequently money inputs are all greatly reduced as a result. It is only used as a batch process at small scales, but when transformed into a continuous extraction process on medium or large scales, it becomes considerably more cheap and feasible.

Table .1 Percentage of yield

Sample	Weight of sample in gms	Weight of the extract	% yield
Benteca rheedi *m*ethanol extract	25 gm	20 gm	80 %

Calculation:

$$\text{Percentage yield} = \frac{\text{Weight of the extract}}{\text{Weight of sample}} \times 100$$

$$= \frac{20}{25} \times 100$$

$$= 80\ \%$$

IN VITRO ANTI-ULCER ASSAY:

ULCER

A skin or mucous membrane ulcer is an open sore that is distinguished by the shedding of inflammatory dead tissue. Lesions on the skin's or mucous membrane's surface known as ulcers are characterized by a superficial loss of tissue.[28,29]

ASSAY:

Principle of assay:

Acid neutralizing capacity

The hydro-alcoholic extract of leaves and bark of Benteca rheedi was tested at different doses (100 mg/ml, 200 mg/ml, 500 mg/ml, and 1000 mg/ml) and its acid neutralizing capacity (ANC) was compared to that of the industry-standard antacid, AHMH (aluminum hydroxide + magnesium hydroxide -500 mg/ml). Water was added and thoroughly mixed

with the 5ml amount of each extract to get the final volume up to 70 ml. Following the addition of 30 ml of 1N HCl and 15 minutes of stirring the standard and test preparation, 2–3 drops of the phenolphthalein solution were added. A 0.5N sodium hydroxide solution was added drop by drop to the excess HCl until a pink tint was visible. [30-38]

The calculation of the moles of acid neutralized includes,

Moles of acid neutralized = (vol. of HCl ×Normality of HCl) - (vol. Of NaOH × Normality of NaOH)

Acid neutralizing capacity (ANC) per gram of antacid = $\frac{\text{Moles of HCl neutralized}}{\text{Grams of Antacid Extract}}$

IN-VITRO COAGULANT ACTIVITY:

Collection of blood samples:- Obtaining blood samples: Using sterile syringes, blood samples from healthy individuals were taken from veins in their right arms and deposited Sseparately in containers containing tri-sodium citrate to stop the clotting process. To acquire pure platelet plasma (ppp) for the prothrombin time test, blood cells were separated from plasma using centrifugation (15 minutes at a speed of 3000 rpm). Each individual's collected plasma sample was pipetted separately into a plane container and kept at room temperature.[39]

Preparation of 25Mm calcium chloride (CaCl2)

To create 0.25 M of calcium chloride (CaCl2), 6.9375g of calcium chloride (CaCl2) is dissolved in 250 ml of distilled water.

Method

At a clean fusion tube, 0.2 ml of plasma, 0.1 ml of crude extract at various concentrations, and various volumes of CaCl2 (25 mM) were combined and incubated at 37 °C in a water bath.

Sodium citrate and ethylenediaminetetraacetic acid (EDTA) were used as references. For the control, the same volume of 0.9% saline water was used in place of the experiment extract solution. By tilting the test tubes every 5 seconds, the clotting time was timed using a stopwatch. The prothrombin time is the name for this period.[40-47]

Statistical analysis :

The resulting experimental data were statistically analysed using Graph Pad Prism. San Diego Trail (Prism Graph Pad Version 8.2.3(263, Graph Pad Software.Inc La Jolla CA.U

RESULTS & DISCUSSION

PHYTOCHEMICAL SCREENING OF METHANOLIC EXTRACT OF *BENTECA RHEEDI*

Table:1 Phytochemical screening results

S. No	Test for	Methanolic extract of Benteca Rheedi
1	Carbohydrates	-
2	Steroids	-
3	Cardiac glycosides	-
4	Steroid glycosides	-
5	Coumarins	-
6	Flavonoids	+
7	Alkaloids	-
8	Tannins	+
9	Terpenoids	-
10	Volatile oils	-

Thin layer chromatography

Rf values of flavonoid compounds identified and their colours on TLC chromatography under the UV light

Table : 2 *Rf* values

Tannins	***Rf* value**	**Color under UV 365 nm**
Orientin	**0.61**	Violet

Figure3 : color under uv light

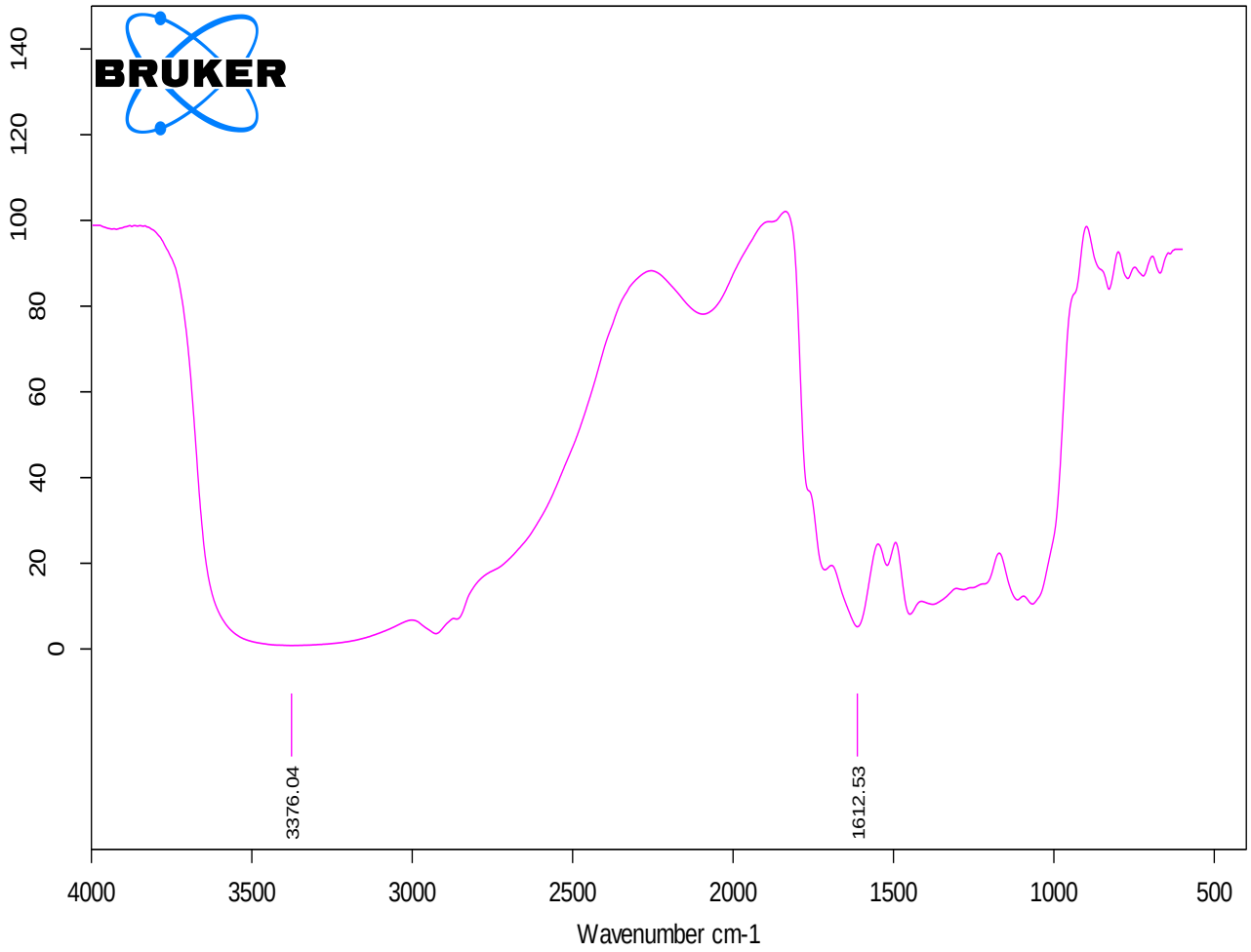


Fig: 3 Graphical representation of IR plant Benteca Rheedi

Table : 3 IR spectra standard values

S.No	Functional group	Standard value	Observed
1.	C=O	1652-1750	1648
2.	OH	600-700	677.7
3.	C-O-C	1150-1070	1086.91
4.	ArH	3100-3300	3348

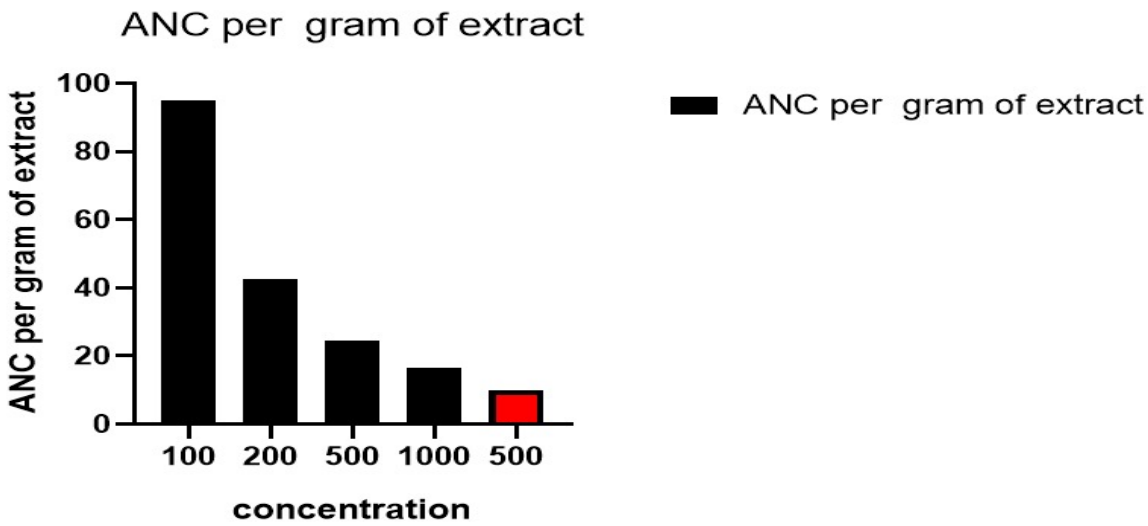
Invitro Anti ulcer effect of Benteca rheedi evaluated by the method

Table:4 Acid Neutralizing Capacity [ANC] of Methanolic extract of Anti-ulcer, Benteca rheedi by *in-vitro* method .

Concentration [mg/ml]	Volume of NaOH Consumed [ml]	mEq of Acid Consumed	ANC per gram of extract
100mg	41	9.5	95
200mg	43	8.5	42.5
500mg	47.7	12.3	24.6
1000mg	26.7	16.6	16.6
$AL(OH)_2$&$Mg(OH)_2$-500 mg	50	5	10

Values are expressed as mean±SEM

The table below shows the Neutralizing effect of various doses of the Methanolic extract of Benteca rheedi on its ability to neutralize acids. Acid neutralizing capacity was demonstrated by the extract (1000 g/ml) and $AL(OH)_2$&$Mg(OH)_2$-500 g/ml.The in-vitro anti ulcer activity of the Benteca rheedi leaves and bark extract employed in our investigation is influenced by AcidNeutralizingCapcity(ANC)



Effect of Coagulant activity of Benteca rheedi

COAGULATION

Table:5 Determination of Coagulation (Pro thrombin Time test) using *Benteca rheedi*

S.No	Concentration (mg/ml)	Extact	Amount of plasma	Amount of Extract	CaCl2	Time
		Control MEBr	0.2 ml	0.1 ml	0.3 ml	70 sec
1.	200	MEBr	0.2 ml	0.1 ml	0.3 ml	85 sec
2.	400	MEBr	0.2 ml	0.1 ml	0.3 ml	1:48 sec

Values are expressed as mean±SEM n= 2trails

Table:6 Determination of Coagulation (Pro thrombin Time test) using *Benteca rheedi*

S.No	Concentration (mg/ml)	Extact	Amount of plasma	Amount of Extract	CaCl2	Time
1.	200	MEBr	0.2 ml	50 µl	0.5ml	2:8 sec
2.	400	MEBr	0.2 ml	100 µl	0.5 ml	3:6 sec

Values are expressed as mean±SEM n= 2 trails

CONCLUSION:

In conclusion, our study provides scientific evidence to support the traditional use of Benteca rheedi in Ayurveda and Unani medicine. The extracts of this plant possess bioactive compounds Exploring the potential of Benteca rheedi exhibit potential anti-ulcer and coagulant activity. Further researchis warranted to find the active constituents responsible for these activities and to evaluate their efficacy and safety in vivo.

On the basis of the results, we may conclude that the Methanolic extract of the species may be considered as a sole source of novel antiulcer and Coagulant drugs. However, a detailed study on the isolation of active constituents from this species and its underlying mechanism of action responsible for its antiulcer and coagulant effect is to be studied in the future.

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