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Evaluation of Antioxidant and Anti-Inflammatory Activity of *Rhynchosia Minima*

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Abstract: Traditionally, medicinal plants have played a significant role on human life since ancient days, at present natural compounds are the major source for the modern drug discovery owing to their therapeutic selectivity minutes of side effects, inexpensive source and serve as lead molecules for the discovery of new drugs. The plant *Rhynchosia Minima* locally known as *KULTA* (Hindi) is an indigenous medicinal plant used traditionally as anti-oxidant and anti-inflammatory, used in the treatment of wounds asthma and piles, extraction of the flavonoids can be performed with solvents that are chosen according to their polarity. The selective crude extract is subjected for isolation by column chromatography by using different solvent. The separation of flavonoids from each fraction by column chromatography was monitored by thin layer chromatography. The pure compounds obtained after separation by the column chromatography were characterized by IR, NMR and Mass Spectroscopy for the structural elucidation.

Keywords: *Rhynchosia Minima*, chromatography, Natural compounds, *Kulta*, Anti-inflammatory, Anti-oxidant.

I. INTRODUCTION

Herbal medicines serve as major remedy in traditional system of medicine, even 21st century these are the primary source of health care system in rural areas and poor countries. According to WHO about 80% of the world populations depend on herbal medicines for primary health care. Herbal medicine practices continue still today because of their biomedical superiority over modern medicine [1,2].

Among the plants of fabaceae family which are used in traditional medicine, *Rhynchosia* species have occupied a prominent role *rhynchosia* genus consists of approximately 300 species circulated throughout the tropical and subtropical areas around the world, out of which 22 species occur in India [3,4]. The plant *Rhynchosia Minima* Synonyms(s) *Dolicholus minimus*, *Dolichos minimus*, *Rhynchosia Minima* Var. *Diminifolia* Family: fabaceae, locally known as Nela Alumu (Telugu) is an indigenous medicinal plant used traditionally as abortifacient, antihelminthic, used in the treatment of wounds, asthma and piles. The seeds are bitter and poisonous and seed extract shows specific agglutinating action on human RBC [3]

Invasive fungal infections (IFIs) are a scourge to human health as they result in high rates of morbidity and mortality. *Candida* and *Cryptococcus* species are the leading causes of these infections. People with compromised immune systems, e.g., organ transplant, HIV/ AIDS and cancer patients, are susceptible to contracting these IFIs, which often lead to life-threatening conditions. [4,5] Proper management of these opportunistic infections requires a swift diagnosis and commencement of antifungal therapy.

Cryptococcus neoformans is a major cause of infectious morbidity and mortality, resulting in an estimated 181,000 deaths every year. [6] *Cryptococcosis*, caused by the fungus *Cryptococcus neoformans*, can be expressed in different forms depending on how it was contracted. Infections may begin in the lungs and spread to other parts of the body such as the central nervous system, urinary system and skin. The pulmonary form often presents in patients with symptoms such as coughing, chest pains, fatigue, skin rash and even bruises. [7]

Excellent progress has been made in diagnosing and treating IFIs, however mortality rates are still high in patients of high risk. [9] Drugs presently used in IFI treatment have limitations in terms of their bioavailability, narrow spectrum and absence of oral or intravenous preparations. [10] There is also a significant spike in antifungal resistance, especially by *Candida* species, which is of great concern to scientists and clinicians. [11,12] Due to these reasons, novel antifungal agents with fewer side effects are needed.

Nature has always been a good source of drugs and lead compounds. [13] Polyenes and echinocandins are two classes of successful antifungal agents that are natural products or their derivatives. [13–14]

II. PLANT PROFILE

A. Taxonomy

- Scientific Name: *Rhynchosia minima*
- Family: Fabaceae (Legume family)
- Common Names: Least snoutbean, Burn-mountainpea
- Synonyms: *Dolicholus minimus*, *Rhynchosianervosa*, *Rhynchosiaphaseoloides*

B. Description

- Type: Perennial herbaceous plant or trailing vine.
- Growth Habit: Prostrate to trailing, occasionally climbing.
- Height: Generally low-growing, less than 1 meter tall.
- Stem: Slender, hairy or glabrous.

C. Leaves

- Type: Compound, trifoliate.
- Leaflets: Three oval to oblong leaflets with smooth or slightly hairy surfaces.
- Size: Leaflets range from 1–5 cm in length.

D. Flowers

- Inflorescence: Raceme or clusters arising from leaf axils.
- Color: Yellow to orange-yellow, sometimes with reddish veins.
- Size: Small flowers, approximately 1–2 cm long.
- Blooming Season: Year-round in suitable climates, but more common during rainy seasons.

E. Fruits

- Type: Small, flattened pods (legumes).
- Size: About 1–3 cm long.
- Seeds: One or two per pod, kidney-shaped, dark brown to black.

III. ENGLISH SYNONYMS

Least snout-bean
Small snout-bean
Dwarf snout-bean

IV. SYNONYMS

Sr.	Languages:	Names:
1.	Afrikaans	Klein-snuithoning
2.	Amharic	tinunish andikiraj
3.	Arabic	al-fasulya al-saghira
4.	Bengali	chhoto shnout bin
5.	French	Haricot à petit museau
6.	German	Kleiner Schnauzenbohne
7.	Hindi	chhota snaut bin

8.	Kiswahili	Koroshon dogo
9.	Malayalam	cheriya snaut bin
10.	Portuguese	Feijão-de-bico-pequeno
11.	Sanskrit	Laghushnouta
12.	Sesotho	Morara o monyenyanane
13.	Spanish	Judía de hocico pequeño
14.	Tamil	chiriya snaut bin
15.	Telugu	chinn snaut bin
16.	Yoruba	Ewe olokun esin
17.	Zulu	Isikhokonke esincane

V. TAXONOMY

Kingdom	plantae
Clade	Angiosperms
Clade	Eudicots
Clade	Rosids
Order	Fabales
Family	Fabaceae
Subfamily	Faboideae
Tribe	Phaseoleae
Subtribe	Phaseolinae
Genus	Rhynchosia
Species	R. minima



Rhynchosia Minima Plant& Their Leaves

V. METHOD OF EXTRACTION- [SOLVENT EXTRACTION]

A. Preparation of Extracts

Take fresh or dried leaves of *rhynchosia minima* plant. After that grind the leaves with the help of mortar and pestle. Then mix with solvent [hexane]. Then allow the mixture to steep for 12 hours and shaking the container occasionally. Then strain the mixture through filter paper. Then evaporate the solvent by using the rotary evaporator or water bath. Then transfer the extract to a clean glass container and store in a cool and dry place.

VI. DETERMINATION OF TOTAL PHENOLICS (TP) CONTENT

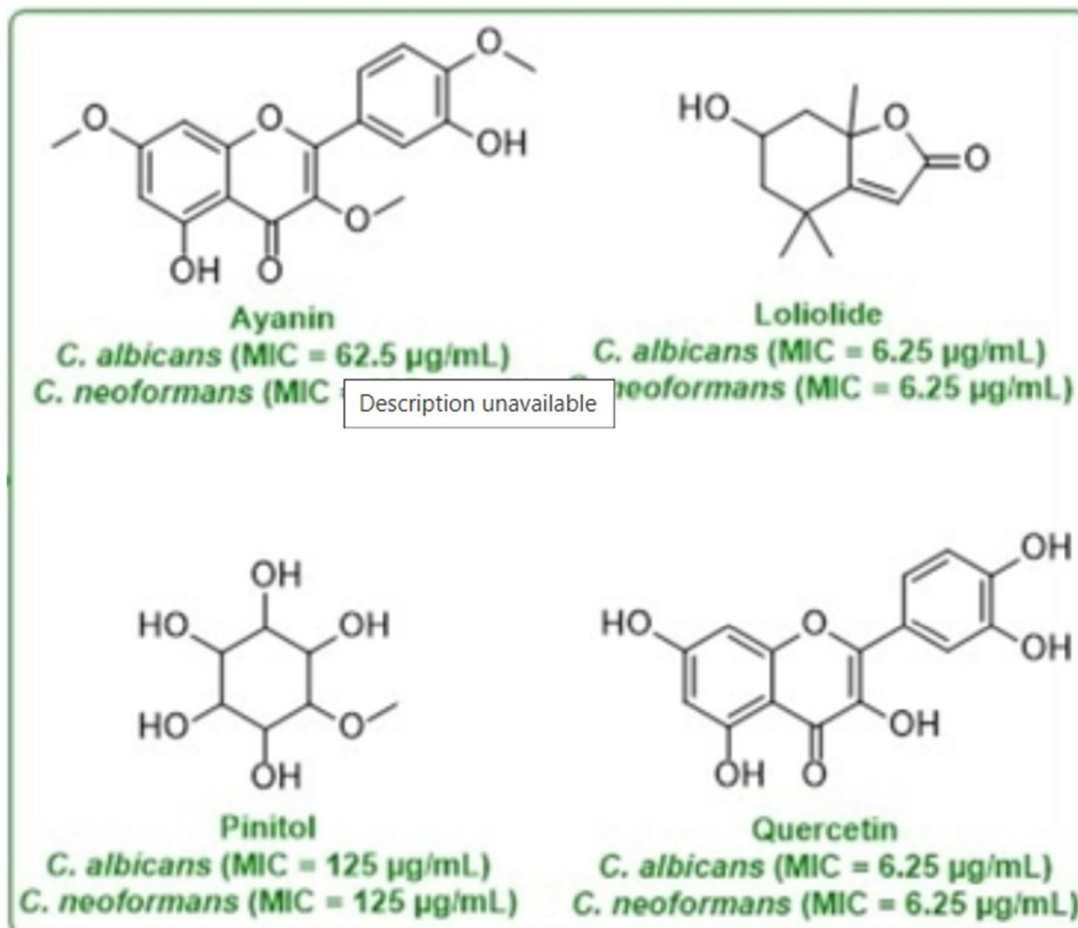
With minimal modification, the Folin–Ciocalteu technique was used to calculate the extracts' total phenolic content¹⁵. In short, 6 ml of distilled water and 100 μ l of Folin–Ciocalteu reagent were shaken for 1 minute with 0.5 ml of diluted extract solution. Following a shake, two milliliters of 15% Na_2CO_3 were added, and the mixture was shaken once more for half a minute. Finally, distilled water was added to the solution until it reached 10 ml. After one and a half hours, a spectrophotometer was used to measure the absorbance at 750 nm. Gallic acid equivalents were used to express the results.

VII. DETERMINATION OF TOTAL FLAVONOID CONTENT

The reported method was used to determine the total flavonoid content of the ethanolic and aqueous extracts of *Rhynchosia minima* (Linn.) DC. *Aluminum* The chloride technique is employed to determine the flavonoid content.¹⁶ Fifty milliliters of distilled water were used to dissolve 25 milligrams of gallic acid. 100 milliliters of pure water were used to dissolve 10 grams of aluminum chloride. Three milliliters of methanol, 0.2 milliliters of 10% aluminum chloride, 0.2 milliliters of 1 M potassium acetate, and 5.6 milliliters of distilled water were combined with one milliliter of the sample (1 mg/ml), which was then left at room temperature for half an hour.

Using a UV-visible spectrophotometer, the absorbance of the reaction mixture was determined at 420 nm. By creating a gallic acid solution (50–150), the calibration curve was extrapolated to calculate the concentration in distilled water ($\mu\text{g/ml}$). Plotting absorbance on the Y-axis and their corresponding concentration on the X-axis allowed for the creation of the calibration curve for gallic acid.

VIII. CHEMICAL CONTITUETNS



A. Qualitative phytochemical screening of aqueous and ethanol extracts of *Rhynchosia minima*

S.No	Plant constituents	Aqueous extract	Ethanol
1.	Alkaloids	+	+
2.	Carbohydrates	+	+
3.	Phenols	+	+
4.	Flavonoids	+	+
5.	Steroids	+	+

B. Traditional uses

Rangaswamy et al., (1974) studied the phytochemistry of seed coat and pericarp and found to contain gallic acid, Hydroquinone diacetate and other phenolics. Elisabeth et al., (1977) studied phenolics and flavonoids in the leaves and reported that all flavonoids of the leaf extract were present in the form of C-glycosylflavones [15]. The hydroquinone present in the seeds of *R. minima* is supposed to be involved in seed germination. Flavonoid profiles of seven species of *Rhynchosia* including *R. minima* were reported by Adinarayana et al., (1985) [16,17]. New flavonoids were identified in the leaf extract of *R. cyanosperma* (Adinarayana et al.,

1980; 1981) [18,19]. In all these studies the medicinal uses of the phytochemical principles were not discussed. However, Gundidza et al., (2009) [20] demonstrated range of 8 essential oils which showed high antibacterial activity against several bacterial and fungal species. N. Yellasubbaiah et al., (2015) studied the anti-oxidant and anthelmintic activity of ethanolic extract of *Rhynchosia minima* [21]. Aqueous, ethanol and ethyl acetate extracts of *R. minima* (Linn) DC were screened against pylorus ligation induced ulcers in rats reported by N. Yellasubbaiah et al., (2017) [22].

IX. PHARMACOLOGICAL PROPERTIES

- 1) ANTIFUNGAL-A previously reported approach was used to assess the extracts' and isolated chemicals' antifungal efficacy. Acetone was used to dissolve the chemicals and crude extracts to a certain degree. of 10 mg/mL and 1 mg/mL, respectively, and a serial dilution was carried out twice using sterile distilled water.[25]
- 2) ANTIBACTERIAL-Antimicrobial drugs stop germs from growing. Numerous chemical substances with antimicrobial properties, including terpenoids, phenolics, peptides, alkaloids, and amines, are produced by plants. (Wink 2013)
- 3) ANTIOXIDANT - DPPH is frequently used to assess a compound's antioxidant activity and determine whether it can function as a hydrogen donor or free radical scavenger.
- 4) ANTI HELMINTHETIC - Albendazole was used as a reference to test the aqueous and ethanol extracts for anthelmintic activity. The anthelmintic assay was conducted using Mathew's technique. [23,24]
- 5) ANTI-INFLAMMATORY- *Rhynchosia minima* contains flavonoids and phenolic compounds that can suppress cytokines like TNF- α and IL-6, prostaglandins (via COX enzymes), and pro-inflammatory mediators like nitric oxide
- 6) Antimicrobial Activity: The essential oil of *Rhynchosia minima* has been demonstrated to exhibit considerable action against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*
- 7) Laxative and Vermifuge Properties: Traditionally, the root of *Rhynchosia minima* has been used as a vermifuge and laxative to treat worm infestations, diarrhea, and dysentery.
- 8) Anti-hemorrhoidal Properties: Hemorrhoids are treated with the plant's root and leaf sap
- 9) Abortifacient Properties: The leaves of *Rhynchosia minima* have been observed to elicit contractions of the uterus, making them potentially helpful as an abortifacient
- 10) Cardiac Activity: It has been discovered that the plant's seed displays heart activity
- 11) Anthelmintic Activity: *Rhynchosia minima* has also been shown to exhibit anthelmintic activity, helping to treat worm infestations

X. CONCLUSION

By using phytochemical screening of methanol and other compounds, the current study demonstrated that the extracts of *Rhynchosia minima* (Linn.) DC *Rhynchosia minima* (Linn.) DC. aqueous and ethanolic extracts include phenols, flavonoids, alkaloids, carbohydrates, and steroids and triterpenoids. Total phenolic and flavonoid content estimation of a methanol extract with high levels of flavonoids and phenolics. Aqueous extract exhibits notable antioxidant activity in DPPH radical scavenging activity and Hydroxyl radical scavenging activity when compared to standard, according to evaluation of in-vitro antioxidant research. Ethanol extract has great activity over the standard in H₂O₂-scavenging activity and significant activity over the standard in the nitric oxide technique.

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