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A Comparative Study of Morphological, Anatomical, Phytochemical and Anti-Inflammatory Activity of Antidesma acidum RETZ. And Antidesma ghaesembilla GAERTN

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Abstract: ***Antidesma is a wild medicinally important genus belongs to Phyllanthaceae family. The leaves and fruits of Antidesma acidum and Antidesma ghaesembiilla are edible as well as medicinal. Both the plants can be used to treat various body ailments. Traditionally, A. acidum is used for muscular pain because of its anti-inflammatory property whereas A. ghaesembilla is used against skin diseases. In the present study, a comparative evaluation of morphological, anatomical, phytochemical and anti-inflammatory activity of A. acidum and A. ghaesembilla is done with several objectives. Both the species have similar morphological and anatomical features with some minute differences. The antioxidant activity of the present study revealed the free radical scavenging property. The antioxidative property of two species of Antidesma genus are compared and found that A. ghaesembilla (73.33%) shows a higher antioxidant activity than A. acidum(15%). The preliminary phytochemical screening reveals the presence of Alkaloid, Flavonoid, Tannin, Polyphenol, Glycoside, Saponin, Protein and Steroid. The quantitative analysis of secondary metabolites reveals that, polyphenol, tannin, glycoside and flavonoid are higher in A. acidum than in A. ghaesembilla. but alkaloid content is significantly higher in A. ghaesembilla. The anti-inflammatory property of species A. acidum and A. ghaesembilla are determined and compared. And the result is concluded with a greater anti-inflammatory property in A. ghaesembilla (28.85%) than A. acidum (23.07%).***

Keywords: Antidesma acidum, Antidesma ghaesembiilla, Antioxidant, Anti-inflammatory, Anatomy, Morphology, Phytochemical

I. INTRODUCTION

Since ancient time plants have been used for the treatment of various ailments. Even today number of important drugs used in the modern system of medicine are obtained from plant. Plants are the treasure house of medicines for most of the diseases seen around. The bioactive compounds present in plants are the reason behind this richness. Different types of secondary metabolites found in the medicinal plants using in curing many kinds of disease and used for manufacturing medicines. So, phytochemicals can be treated as standards which can be used for the systematic drug identification. Morphological as well as anatomical features can also be set as standard for the proper identification of drug.

Phyllanthaceae is a family belongs to order Malpighiales. Phyllanthaceae include in the list of most important medicinal families. Both the subfamilies, Antidesmatoideae and Phyllanthoideae has medicinal value. These medicinal plants contain certain organic compounds such as alkaloid, tannin, flavonoid, carbohydrates, terpenoids, steroids which produce definite physiological action on the human body. Secondary metabolism produces many specialized compounds that do not aid in the plant growth and development, but plant required it to survive in its environment. They are widely used in pharmaceutical field. Many phytochemicals belonging to several chemical classes have been shown to have anti-inflammatory, anti-diabetic, anti-cancerous, anti-microbial effects etc.,

Antidesma acidum and Antidesma ghaesembilla are two wild shrub belongs to Phyllanthaceae. Traditionally, the leaves of A. acidum is used to treat muscular pain, stomachache and the leaves of A. ghaesembilla have been used in traditional medicine for treatment of skin disease and headache. A. acidum have anti- inflammatory property. Both the plants belong to same genus Antidesma, with almost similar morphological and anatomical characters brings the problem of difficulty in the process of identification. In such a situation the proper identification of plant and drug have importance in the determining the purity of the drug. So, the morphological, anatomical and phytochemical standards have the role in maintaining the purity and safety of the drug.

II. MATERIALS AND METHOD

A. *Source Of Plant*

Fresh leaves of *Antidesma acidum* is collected from a Hill of a rural locality of Malappuram district of Kerala. It is collected from laterite soil, where the average annual rainfall is 2952mm. Leaves of *Antidesma ghaesembilla* is collected from Malabar Botanical Garden and Institute for plant sciences at Kozhikode, Kerala. Where the soil is laterite type, and the average annual rainfall is 3205mm.

B. *Morphological and Anatomical Analysis*

Morphological parameters of *A. acidum* and *A. ghaesembilla* leaves were studied by their physical characters. Anatomical features like internal cellular structure, stomal index, number of stomata, trichome are analyzed under Compound Microscope and Scanning Electron Microscope (SEM). Stomatal index per unit area is calculated using the formula:

Stomata index (SI)= $\frac{S}{S+E} \times 100$

S- Number of stomata per unit area

E- Number of epidermal cells per unit area

C. *Extract Preparation*

The collected leaf samples were shade dried separately. Dried leaves were finely powdered by using a blender. Further studies were done by using these dried leaf powder. 10g of dried leaf powder were subjected to methanol extraction using Soxhlet apparatus at the temperature of 70°C for 24hrs. The extract was dried by Vacuum evaporator to obtain dry extract. The extract was used for advanced studies.

D. *Qualitative Analysis (Yadav and Agarwala, 2011)*

The plant extracts were subjected to qualitative phytochemical analysis by using various tests for alkaloids, carbohydrates, flavonoids, glycoside, phenol, protein, saponins, steroids, tannins, and terpenoids.

1) *Test for Alkaloids:* 2ml of extract was treated with a few drops of dilute hydrochloric acid. Then 1ml of Dragendorff‟s reagent was added. The presence of alkaloids was confirmed by appearance of orange to red precipitate.
2) *Test for Tannins:* To 2ml of each extract a few drops of 10% lead acetate were added. The white colored precipitate indicates the presence of tannins.
3) *Test for Phenol:* To 2ml of each extract a few drops of aqueous ferric chloride is added. The formation of a blue, violet, purple, green or red color upon addition indicates the presence of phenol
4) *Test for Saponins:* To 1ml of extract taken in a measuring jar, 9ml of distilled water was added and shaken vigorously for 15seconds and extract could stand for 10min.Formation of stable foam (1cm) indicates the presence of saponins.
5) *Test for Steroids:* 10ml Chloroform was added to 2ml of all plant extracts. To these extracts 1ml acetic anhydride was added and then 2ml concentrated sulphuric acid was added along the sides of the test tube. Color formation at the junction is noted. The presence of steroids was confirmed by the blue green color appearance.
6) *Test for Triterpenoids:* The test for Triterpenoids is like steroids, so, the presence of Triterpenoids is confirmed by the appearance of red, pink color or violet color at the junction.
7) *Test for Glycosides:* To 1ml of each extract a few drops of glacial acetic acid and ferric chloride and 3-4 drops of concentration sulphuric acid were added. The presence of glycosides was confirmed by the appearance of blue-green color.
8) *Test for Flavonoid:* 4ml of extract solution was added to 1.5ml of methanol solution. The solution was warmed and treated with metal magnesium. Then 5-6 drops of Con. HCl acid were added, and an orange color was observed for flavonoids.
9) *Test for Carbohydrates:* Fehling A and Fehling B reagents were taken equally and mixed. Then 2ml of it was treated with crude extract and gently boiled. The presence of reducing sugars is indicated by a brick red precipitate appeared at the bottom of the test tube.
10) *Test For Proteins:* Crude plant extract is treated with 2ml of Millons's reagent. The presence of protein was confirmed by a white colored precipitate was formed which turned red upon warming.

E. *Quantitative Analysis*

The phytochemical studies were carried out by quantitative analysis of total polyphenol, total tannin, glycoside, alkaloid and flavonoid of both species of *Antidesma.*

F. Determination of Total Polyphenol Content (Folin-Ciocalteu method) (Singh et al., 2007).

20µl plant extract is treated with 6.980ml distilled water, 2ml Sodium carbonate (Na_2CO_3) and 0.8ml Folin's reagent. And is allowed for incubation at 2 hours. Then the optical density is measured at 765nm. The control contains 7ml distilled water, 2ml Na_2CO_3 and 0.8ml Folin's reagent.

$$\% \text{ of polyphenol} = \frac{OD\ of\ test\ sample}{OD\ of\ standard} \times \frac{concentration\ of\ standard}{volume\ of\ test\ sample}$$

G. Determination of Total Tannin Content (Folin-Ciocalteu method) (Polshettiwar et al., 2007)

20µl plant extract is added with 980 µl of distilled water and 4.5ml Na_2CO_3. Then it can stand for 10 minutes. The add 0.5ml Folin's reagent and a 30 minutes incubation. The optical density is measured at 725nm. The control is prepared by treating 1ml distilled water with 4.5ml Na_2CO_3. Then it can stand for 10 minutes. The add 0.5ml Folin's reagent and a 30 minutes incubation.

$$\% \text{ of Tannin} = \frac{OD\ of\ test\ sample}{OD\ of\ standard} \times \frac{concentration\ of\ standard}{volume\ of\ test\ sample}$$

H. Determination of Total Glycoside Content (Balget's test) (Balbaa et al.,1981)

1ml of plant extract is treated with 1ml freshly prepared Balget's reagent (95ml 1% Picric acid + 5ml 10% NaOH). Incubated for one hour. After incubation diluted with 10ml distilled water and the absorbance was read by Spectrophotometer at 495nm.

$$\% \text{ of Glycoside} = \frac{absorbance}{17} \times 100$$

I. Determination of Total Alkaloid Content (Harborne method- 1973)

5-gram of plant extract is treated with 30ml 10% Glacial Acetic acid is covered and allow to stand for 5 hours. Sample is filtered, concentrate on water bath to get 1/4 of its original volume. Then add 10ml of concentrated Ammonium hydroxide dropwise with continues stirring until the precipitate was complete. All the solution can settle. Collect the precipitate and washed with diluted Ammonium hydroxide (5ml ammonium hydroxide + 5ml water) and the filtered through a pre-weighed filter paper. The residue was dried and weighed.

$$\% \text{ of alkaloid} = \frac{\text{final weight} - \text{initial weight}}{\text{weight of sample}} \times 100$$

J. Determination of Total Flavanoid Content (Bohm and Kocipai-Abyazan method- 1994, Cameron et al., 1993)

5gram of plant extract is treated with 30ml 80% methanol. It is covered and allowed to stand constantly for 2 hours. The solution was filtered through the Whatman filter paper No:42. The filtrate was transferred into a crucible(pre-weighed) and evaporated into dryness and weighed to a constant weight.

$$\% \text{ of flavonoid} = \frac{\text{final weight} - \text{initial weigh}}{\text{weight of sample}} \times 100$$

K. IN-VITRO Antioxidant Studies

Antioxidant activity by DPPH method (2,2-Diphenyl-1-picrylhydrazyl) (Blois, 1958)

DPPH radical scavenging protocol was using for estimation of antioxidant activity of the plant extracts. DPPH solution (0.004% w/v) was prepared in 95% ethanol. 1 ml of freshly prepared DPPH solution (0.004% w/v) was added in each of these test tubes contains 1 ml extract. The reaction mixture was incubated in the dark for 30 min and thereafter the optical density was recorded at 523 nm against the blank. 1 ml of DPPH solution in ethanol was mixed with 10ml of ethanol and the optical density of the solution was recorded after 30 min was considered as control. The assay was carried out in triplicate. The decrease in optical density of DPPH on addition of test samples in relation to the control was used to determine the antioxidant activity, as percentage inhibition (%IP) of DPPH radical. The capability of scavenging DPPH radical was calculated using the following equation

$$\% \text{ of Antioxidant activity} = \frac{Abs\ Control - Abs\ sample}{Abs\ control} \times 100$$

L. IN-VITRO of ANTI-INFLAMMATORY Activity

Inhibition of Albumin denaturation

The anti-inflammatory activity of *Antidesma acidum* and *Antidesma ghaesembilla* was measured by using inhibition of albumin denaturation technique which was carried out according to Mizushima and Kobayashi (1968) and Sakat *et al.* (2010) followed with minor modifications. The reaction mixture was consisting of 1ml test extracts and 1ml of 1% aqueous solution of Bovine albumin.

The sample extracts were incubated at 37°C for 20min and then heated to 51°C for 20min, after cooling the samples turbidity was measured at 660nm (UV Visible Spectrophotometer). The experiment was performed in triplicate. The control mixture was consisting of 1ml of standard anti- inflammatory drug (Aceclofenac) and 1ml of bovine albumin.

The percentage of inhibition of protein denaturation was calculated as follows.

$$\% \text{ of inhibition} = \frac{Abs\ Control - Abs\ sample}{Abs\ control} \times 100$$

III. RESLTS AND DISCUSSION

The present study was carried out on Morphological, Anatomical, Phytochemical analysis and Anti-inflammatory activity of *Antidesma acidum* Retz. and *Antidesma ghaesembilla* Gaertn., Traditionally, *A. acidum* was employed in the anti-inflammatory activity. In order to know the activity of both species scientifically present study were carried out.

A. Morhological Description

Antidesma acidum is a large shrub of about 3-5m tall. The shoot is glabrous. Leaves are simple, alternate, petiolate. Leaves 3-9×2-4cm, broadly elliptical or orbicular obovate, base attenuate, apex shortly acuminate. Petiole is usually short length up to 3mm long. Margin is entire and pubescent. Base rounded more rarely obtuse, apex mucronate (fig i).

Antidesma ghaesembilla is a small medium tree up to 20m tall, young twigs pubescent. Leaves are simple, alternate and shortly petioled. Leaves oblong, more rarely ovate or obovate, papery to thinly leathery, pubescent to glabrous especially adaxially. Base rounded to cordate, rarely obtuse, apex rounded, more rarely obtuse or acute, sometimes mucronate or retuse (Fig iv).

Fig i: Habit of *A. acidum*

Fig ii: Habit of *A. ghaesembilla*

Fig iii: Leaf of *A. acidum*

Fig iv: Leaf of *A. ghaesembilla*

B. Anatomical Parameters

The internal structure of both *Antidesma acidum* and *Antidesma ghaesembilla* are similar. The difference occurs in the shape of midrib of the leaf. The midrib of *Antidesma acidum* is almost rounded at the margin whereas in *A. ghaesembilla* rectangular shape with slight curve at the edge. In the transverse section of leaf, the dorsal and ventral epidermis is made up of compactly arranged, single layer of hexagonal shaped cells. Epidermis lacks chloroplast. Epidermis consist of large number of unicellular non-glandular trichomes present in both the species of *Antidesma*. Outer surface of the epidermis is covered with cuticle and paracytic stomata are scattered on the ventral surface of leaf of both species of *Antidesma*.

Leaf lamina differentiated as parenchymatous cells with several chloroplast and large intercellular space. Mesophyll cells are differentiated into upper palisade parenchyma and lower spongy parenchyma. Upper palisade parenchyma is composed of compactly arranged 2-4 layered elongated columnar cells whereas lower spongy parenchyma cells are composed of loosely arranged, spherical shaped cells with large intercellular space. Palisade parenchyma cells of mesophyll contain numerous chloroplasts. The above characters were observed in both species of *Antidesma*.

The upper and lower epidermis of leaf blade continues up to the midrib region. In the T.S of midrib, epidermis is followed by collenchyma cells at both upper and lower side. Sclerenchyma cells are present just below the stelar region. Vascular bundles are conjoint, collateral and open. Xylems are endarch. Pith is represented by parenchymatous cells.

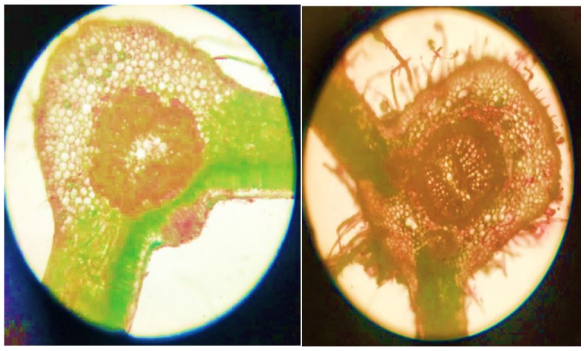


Fig v & vi : Anatomy of *A. acidum* and *A. ghaesembilla*

C. Stomata

The stomatal characters of both species of *Antidesma* were studied. In the present study, it is identified that paracytic stomata was present in both species. They were identified by the presence of one or more subsidiary cells parallel to the opening between the guard cells. However, *A.ghaesembilla* shows the presence of paracytic stomata in their ventral surface of leaves but they were totally absent in dorsal surface. The structure of stomata and their characters were identified by SEM(Scanning Electron Microscope) (fig ix). The stomatal index of *A. acidum*(24.62) is slightly higher than that of *A. ghaesembilla* (23.73). The area of stomata was also measured to identify both the species. In which area of stomata was found to be higher in the species of *A.acidum* (344.567μm^2) when compared to *A.ghaesembilla* (216.113 μm^2). The ultrastructure of stomatal characters was represented in the Table-I.

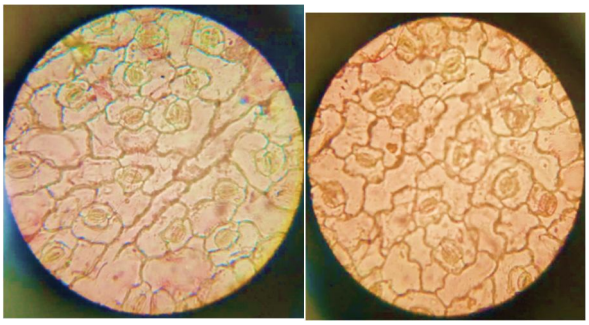


Fig vii &viii: Stomata of *A. acidum* and *A. ghaesembilla*

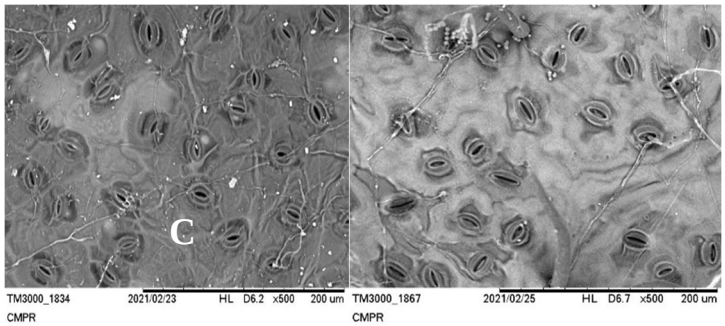


Fig ix (a) Fig ix (b)

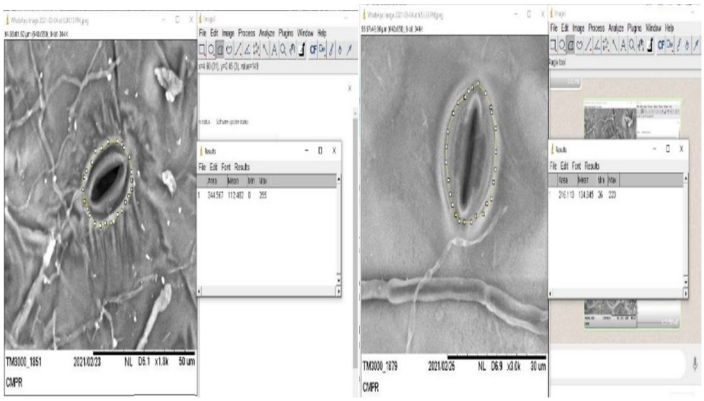


Fig ix (c) Fig ix (d)

Fig ix: Stomata of *A. acidum* and *A. ghaesembilla*

D. Trichome

Unicellular and long trichomes are present on both upper and lower side of the leaf in both *Antidesma acidum* and *Antidesma ghaesembilla* species. The structure of trichomes were studied by SEM to know the major difference which helps to identify both the species. Two measurements were considered, the length and Area. In which area and length was gradually higher in *A.ghaesembilla* (103.437 µm^2 and 234.237 µm) compared to *A.acidum (*305.891 µm^2 and 342.912 µm) (Fig x and xi).

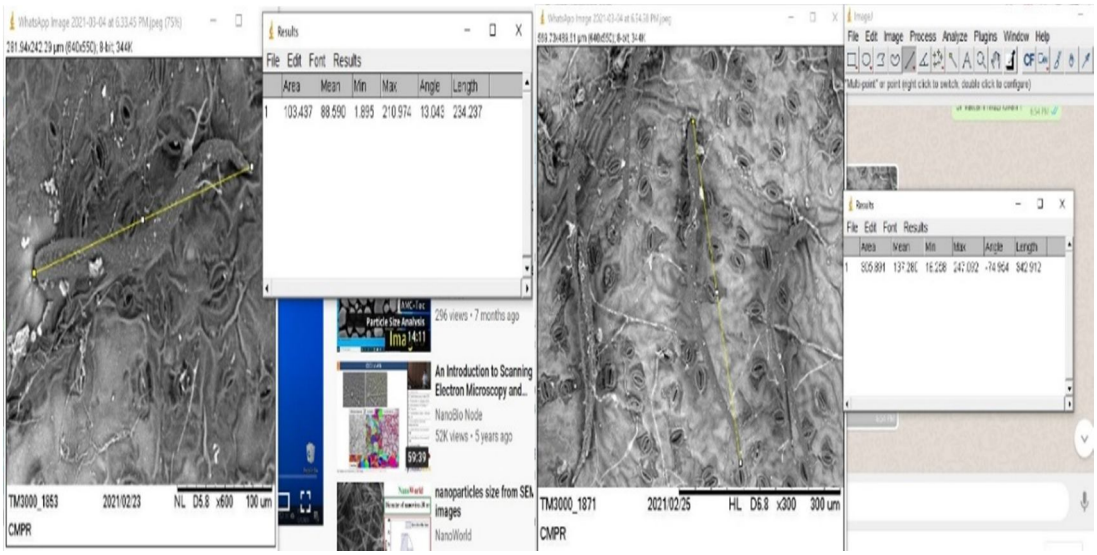


Fig x : Trichome of *A. acidum* Fig xi: Trichome of *A. ghaesembilla*

TABLE I: Anatomical analysis of *A.acidum* and *A.ghaesembilla*

Plant species	Trichome		Stomata	
	Area (µm^2)	Length (µm)	Stomatal index	Area (µm^2)
Antidesma acidum	103.437	234.237	24.62	344.567
Antidesma ghaesembilla	305.891	342.912	23.73	216.113

E. Phytochemical Parameters

The results show that the presence almost all phytochemicals such as Alkaloid, Flavonoid, Tannin, Polyphenol, Glycoside, Saponin, Protein and Steroid except Carbohydrate and Triterpenoid.

Table II: Preliminary phytochemical analysis of methanolic extract of *A.acidum* and *A.ghaesembilla* leaf.

Phytochemical constituents	*A.acidum*	*A.ghaesembilla*
Alkaloid	++	+++
Flavonoid	+++	++
Tannin	++	+
Polyphenol	+	+
Glycoside	++	++
Saponin	+	+
Protein	+	+
Carbohydrate	-	-
Steroid	+	+
Triterpenoid	-	-

+ indicates the Presence
- indicates the Absence

From the tests conducted the in both plants' methanol extract showed the presence of phytochemicals like alkaloids, flavonoids, tannin, glycosides, polyphenols, saponin, protein and steroid whereas carbohydrates and triterpenoids are totally absent. Among these phytochemicals Alkaloids and flavonoids are present in higher concentration than the others.

F. Antioxidant Property (DPPH Scavenging Assay)

DPPH scavenging assay was performed in both species of *Antidesma* using methanol extract. In which *A.ghaesembilla* (73.33%) shows higher DPPH free radicals scavenging activity when compared to *A.acidum* (15%). The result of the antioxidant activity of DPPH was represented in Figure I. DPPH scavenging activity of both species of *Antidesma* shows the activity in dose dependent manner.

G. Total Polyphenol, Tannin Glycoside, Alkaloid and Flavonoid Content

Various parameters such as polyphenol, tannin and glycoside content were determined for both the species of *Antidesma* by using various methods. The total phenol, tannin and glycoside content was found to be higher in *A. acidum* compare to the methanol extract of *A. ghaesembilla.* The content of total polyphenol, tannin and glycoside in *A.acidum* were 0.182, 2.188 and 3.94 respectively. Total alkaloid and flavonoid content were tested for both *A. acidum* and *A. ghaesembilla.* The alkaloid content was found to be higher in *A. ghaesembilla* (12.2%) and flavonoid content was found be higher in *A. acidum* (7.52%) (TABLE-3).
Glycoside content was found to be higher in *A. acidum* and Alkaloid was much higher in *A. ghaesembilla* compared to *A. acidum*

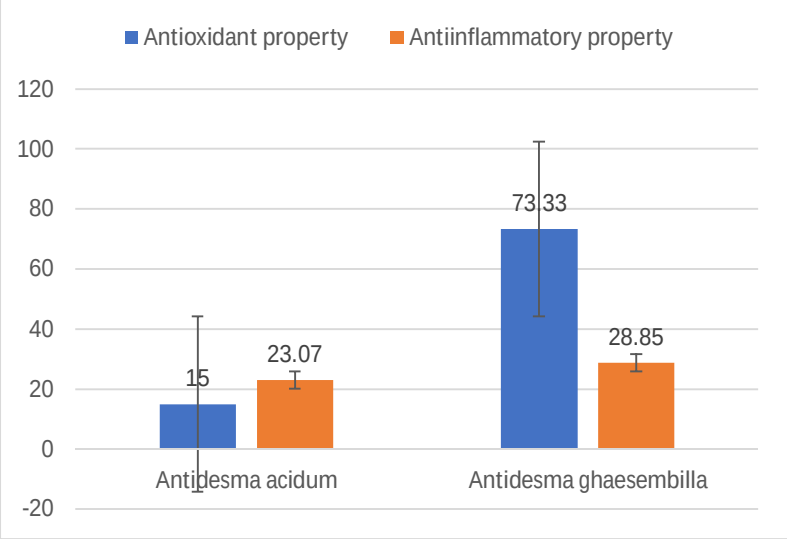
TABLE III: Percentage of total polyphenol, tannin, glycoside, alkaloid and flavonoid content in *Antidesma acidum* Retz than *Antidesma ghaesembilla* Gaertn.

PARAMETERS	*Antidesma acidum*	*Antidesma ghaesembilla*
Polyphenol(%)	0.182± 0.02	0.117± 0.01
Tannin(%)	2.188± 0.03	1.7201± 0.2
Glycoside(%)	3.94± 0.31	3.64± 0.11
Alkaloid (%)	2.56± 1.2	12.12±5.7
Flavonoid (%)	7.52± 0.89	5.78± 1.1

H. Anti-inflammatory Property

The anti-inflammatory activity of methanol extract of both species *A. acidum* and *A. ghaesembilla* was tested in terms of its ability to inhibit denaturation of albumin. The maximum inhibition was observed in *A. ghaesembilla* by preventing albumin denaturation in concentration dependent manner. Thus, *A. ghaesembilla* showed anti-inflammatory activity when compared to *A. acidum.* The percentage of protein inhibition is 23.07 and 28.85% for *A.acidum* and *A. ghaesembilla* respectively (Figure-XII).

Figure XII: Percentage of antioxidant and anti-inflammatory property in *Antidesma acidum* Retz than *Antidesma ghaesembilla* Gaertn.



IV. CONCLUSION

In conclusion, it is apparent that the pharmacological activities of Antidesma acidum Retz and *Antidesma ghaesembilla Gaertn* reflects its uses in traditional medicine. The plant showed significant antioxidant and anti-inflammatory activities with very low toxic side effects. Thus, present investigation serves as standard reference for identification and distinguishing the A. acidum Retz and *A. ghaesembilla Gaertn* leaves from its substituent and adulterants and consequently, the isolation of bioactive compounds from this plant might be our future research. The detailed and systematic Pharamacognostical evaluation would give valuable information for the future studies.

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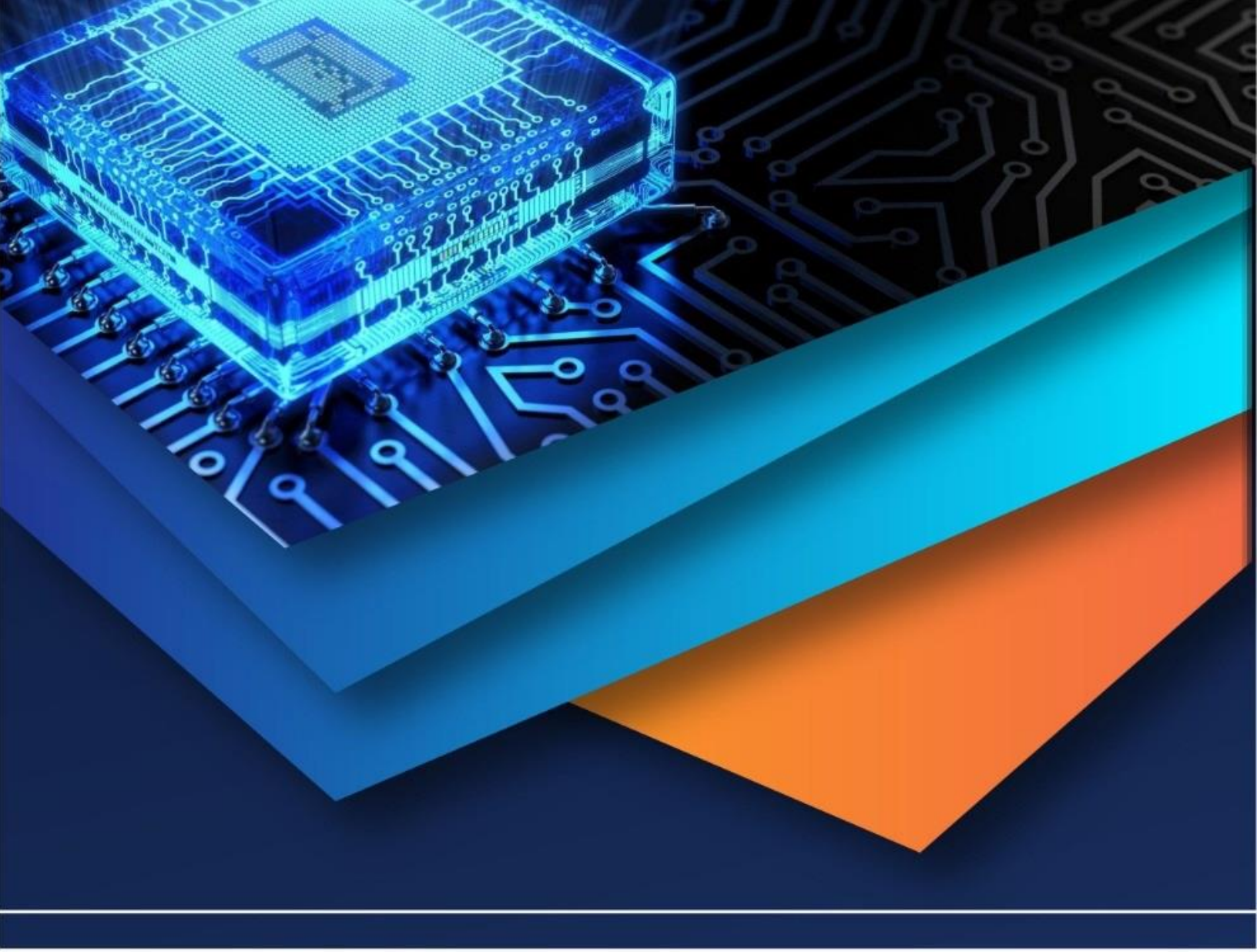

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