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PHARMACOGNOSTICAL, PHYTOCHEMICAL SCREENING, INVITRO ANTI-OXIDANT AND ANTI-BACTERIAL ACTIVITY OF *Klenia grandiflora*

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Abstract

Objective: A hydroalcoholic extract of *Klenia grandiflora* leaves was evaluated for its anti-microbial and anti-oxidant properties. Most areas with a warm climate have a large variety of *Klenia grandiflora*, which are popular ornamental plants.

Methods: A hydroalcoholic extract of *Klenia grandiflora* (leaves) was tested for anti-microbial activity by observing the Minimum Inhibitory Concentration zone of inhibition against both gram- positive and gram-negative bacterial strains, including *Staphylococcus aureus*, *Bacillus substilis*, and *E. coli.* Agar cup plates were used to study antibacterial activity, and hydrogen peroxide free radical scavenging was used to assess antioxidant activity.

Results: Inhibitory zones were observed against gram negative as well as gram positive bacteria by the hydroalcoholic extract of *Klenia grandiflora*. The anti-microbial effects of Gentamicin standard showed inhibitory effect on *E. coli, Staphylococcus aureus and Bacillus substilis* extremely low concentration. *Klenia grandiflora* hydroalcoholic extracts were found to inhibit H_2O_2 scavenging activity by a significant amount compared to ascorbic acid in the study. Several antimicrobial and antioxidant activities of the plant were found in this study.

Conclusion: Discovering metabolites from *Klenia grandiflora* may lead to the development of anti-biotics and anti-oxidants. It may be possible to identify antimicrobial and antioxidant metabolites in *Klenia grandiflora* by exploring its potential.

Keywords: *Klenia grandiflora*, Anti-oxidant, Anti-microbial, Extracts, Drugs

Introduction

The common name of the plant is large flower Klenia, the botanical name of *Klenia grandiflora* in the family of Asteraceae (sunflower family), the other names of *Notonia grandiflora*, *Senecio indicus*. Large flowers are found on *Klenia grandiflora* plants, whose stems are blackish brown and adorned with prominent leaf scars. A sparse amount of branching is found in the upper portion of the stem, which is hairless and glaucous. Inverted lance-shaped blackish brown leaves have blunt tips and are elliptic-oblong in shape. In addition to being hairless and glaucous, they have a slightly stalked base and have a narrowed shape. Corymbs or relax-branched panicles, measuring 15-20 inches long, contain bell-shaped flower heads measuring 3-6 inches in height. There is a broad lance-shaped pale green bract with a striped appearance that is sub membranous and has purplish margins. The medicinal uses of large flower Klenia used as a cure for pimples and as a remedy for hydrophobia. [1]

This study investigates the corrosion inhibitor properties of the Asteraceae plant *Klenia grandiflora*. As a versatile plant, it can be used for a variety of ailments, such as treating Joint pain, pimples, alleviating allergies, cough and cold, bacterial inspection, issues in urinary system, as well as aiding digestion. Laxative properties have also been reported for it. [2]

In addition to providing primary healthcare to millions of people, plants serve a variety of other purposes in daily life and could serve as a potential source for novel pharmaceuticals. A wide range of illnesses have been treated with plants around the world for centuries, in various forms. Globally, 70-80% of the population relies on non-conventional medicine for their health needs, particularly in developing countries. The rich variety of phytochemicals in medicinal plants found in India, thanks to its abundant and widely distributed flora, causes them to exhibit diverse pharmacological effects. It is still unclear whether these plants are effective despite their widespread use. [3]

The purpose of this study was therefore to scientifically validate the traditional knowledge regarding *Klenia grandiflora* leaf extract's antipyretic properties. In addition, preliminary phytochemical analyses and acute toxicity studies were conducted on the plant extracts to provide additional insights into their composition and safety. [4][5]

Methodology

Collection and Authentication of Plants

Plants were collected from Perambur village in Pudukkottai District in November 2022 and authenticated by Professor D. Stephen at American College, Madurai-20. We have preserved a herbarium of this specimen for future

reference.

Macroscopy

Organoleptic characteristics of the plant, such as color, odor, taste, shape, and other characteristics, were thoroughly examined. These attributes were analyzed macroscopically.

Microscopy

To section the material, follow these steps:

Make sure you have a firm grip on the material with your left thumb and forefinger. Carefully drag the razor across the object in quick, sequential motions with your right hand. Make sure your forefinger is pointing toward you while you slide the razor over your forefinger. The material will be cut into thin sections using this technique. In a watch glass filled with water, place the sections once you have made them. The sections should be delicately moved from the razor to the water with a camel's hair brush. A different approach can be used if you are working with tender and flexible materials, such as fresh leaves or tubers. Make a longitudinal slit about 2cm deep in a piece of pith, typically a potato. A slit should be aligned with the flat surfaces of the pith, between which the material to be sectioned is to be placed. The sections should be cut in the same manner as described earlier. Make use of a camel's hair brush to transfer the sections into the water.

Physico-Chemical Parameters

Foreign organic matter determination

It was calculated that 100g of air-dried coarse drug weighed exactly 100 grams. In order to achieve an even distribution of the drug sample, a thin layer was applied evenly. A 6x microscope or naked eye was used to visually inspect the sample. A thorough separation of foreign organic matter was performed manually on the drug sample. A careful weight was taken of the organic matter that was separated. Weights of the separated foreign matter were divided by the weights of the drug taken (100g) to calculate the percentage of foreign organic matter. These steps provided valuable information about the quality and purity of the drug sample since a percentage of foreign organic matter could be determined.

Moisture content identification (Loss on drying)

An accurate weight of 10g of the coarsely powdered drug was measured. The drug was placed in a tarred evaporating dish. The dish, along with the drug, was dried at a temperature of 105°C for a duration of 3 hours. After the initial 3-hour drying period, the dish was removed from the oven and allowed to cool. The dish, now containing the dried drug, was reweighed to determine the weight after drying. The drying and weighing process was repeated at one-hour intervals until the difference between the two successive weighing was not more than 0.25%. Once the desired level of consistency was achieved, the final weight of the dish and dried drug was recorded. The loss on drying was calculated by subtracting the final weight from the initial weight, and expressing it as a percentage of the initial weight (10g).

Extractive value (alcohol extractive value) determination

Take out 5g of air-dried and coarsely powdered drug. In a closed flask, place the drug and 100ml of alcohol that has a specified strength. For the first six hours, shake the flask frequently and then let it stand for 18 hours to allow it to macerate. In order to prevent any loss of solvent, filter the mixture quickly following the maceration period. A shallow tared dish with a flat bottom will work well for evaporating 25ml of filtrate. In order to determine the weight of the extractive residue, the dish is weighed once the solvent has completely evaporated. Once all the solvent has evaporated, repeat the drying and weighing process until you reach a constant weight. Multiply the weight of the extractive residue by the weight of the air-dried drug to determine the percentage of alcohol-soluble extractive.

Water soluble extract

For determination of alcohol soluble extractives, proceed as directed using water rather than ethanol.

Ash value determination

Total ash

The air-dried coarsely powdered drug should be weighed accurately to the nearest 3g. Weigh the drug sample and place it in a silica crucible with tar on it. Incinerate the crucible and its contents at a temperature not exceeding 450°C until all carbonaceous materials have been burned and the crucible is carbon-free. Combust thoroughly. Once the crucible and residue have reached room temperature, remove them from the oven. After weighing the crucible with residual ash, determine its weight. Subtract the weight of the crucible with ash residue from the weight of the crucible with ash residue to calculate the percentage of ash. By multiplying this difference by the weight of the air-dried drug (3g), you obtain the difference in weight. [6][7]

Extract preparation

A defatting process using petroleum ether (60-80°C) was applied to shade-dried leaves of *Klenia grandiflora*. Following defatting, the remaining plant material, or marc, was extracted using a Soxhlet extraction apparatus using a hydroalcoholic solution (70% ethanol). A continuous extraction process was carried out until the material had been completely extracted. A solid residue was obtained by concentrating the resulting extract under reduced pressure to remove the solvent. A dark green color was exhibited by the solid residues resulting from the concentration process.

[8]

Phytochemical screening

An analysis of the qualitative chemical composition of *Klenia grandiflora* hydroalcoholic extract was conducted. Anathraquinone glycosides, alkaloids, phenolic compounds, flavonoids, saponins, and steroids were all detected in the extract via various chemical tests. To determine whether these chemical constituents were present in the extract, these tests were conducted. Based on the chemical class being investigated, specific methods were used for each compound. Each compound's presence or absence was determined by observing and analyzing the results of the tests. Performing the qualitative chemical analysis of *Klenia grandiflora* hydroalcoholic extract provided

valuable information regarding its phytochemical composition. Analyzing the plant extract revealed the presence of anthraquinone glycosides, alkaloids, phenolic compounds, flavonoids, saponins, and steroids, providing insight into the potential bioactive constituents.

Identification test for anthraquinone glycosides

Borntrager's test

A dilute sulfuric acid solution was used to boil the powdered drug. To obtain a filtrate, the mixture was boiled and filtered. A vigorous shake was used to mix benzene with the filtrate. It was separated from the aqueous layer by the organic layer, which contained the benzene and any dissolved compounds. A slow addition of ammonia solution was made to the organic layer. As the ammonia solution was shaken well, the organic compounds in the mixture were allowed to react. Anthraquinone in the original powdered drug caused an ammoniacal layer to turn pink, violet, or red.

Determination of Alkaloids

1. Mayer's test

One ml of extract was diluted with a few drops of Mayer's reagent. There was a creamy white precipitate formed.

2. Dragendroff's test

Extract was mixed with Dragendorff's reagent in a volume of 1ml. Precipitate formed in the form of an orange red color.

3. Hager's test

Several drops of Hager's reagent were added to 2ml of extract. Precipitate formed in a yellow color.

Identification of phenolic compounds

1. **Ferric chloride test**

The powdered drug was extracted with water in small quantities. We added a few drops of ferric chloride solution to the alcoholic extract. It was observed that the colour was bluish black

Identification test for flavonoids

1. **Shinoda's test**

Using alcohol to heat the powder, a small amount of the drug was filtered. Magnesium turnings were added to the test solution, along with concentrated hydrochloric acid a few drops at a time. After boiling for five minutes, remove from heat. The colour obtained was red.

2. **Lead acetate test**

Adding a few drops of 10% lead acetate to the test solution will give a positive result. White precipitate is produced.

3. **Acid test**

Yellow orange colour was obtained from the small amount of test solution when a few drops of concentrated sulfuric acid were added. This indicates flavonoids are present in the solution.

Identification of saponins

The presence of saponins was determined by vigorously shaking 0.1g of powder with 5ml distilled water for 30 seconds and leaving it undisturbed for 20 minutes.

Identification of sterols

1. **Salkowski's test**

In a test tube, concentrated sulfuric acid was added along the walls after the test extract was shaken with chloroform. The presence of steroids was indicated by the appearance of a red color. [9] [10]

Anti-Bacterial Activity

Agar-nutrient medium

We prepared a culture medium by mixing beef extract, sodium chloride, peptone, and agar with 200ml of distilled water. Dissolution was ensured by heating the mixture until it boiled. After autoclaving for 15 minutes at 121°C at 15 lb/in^2 pressure, the medium was sterilized at 15 lb/in^2. Surgically sterilized glass Petri dish plates were used to transfer the medium after sterilization. In order for the medium to solidify, the plates were left at room temperature for several hours. Each Petri plate was then inoculated with 15g of bacteria. We created four wells in the medium with a diameter of 6mm each using a sterile borer. In the next step, different concentrations of drug samples were added using sterile micropipettes. To allow the drug solution to diffuse into the medium, the plates were left undisturbed for two hours. Petri dishes were subsequently inverted and placed in an incubator at 37°C±1°C for 24 hours. It was measured and recorded the diameter of the inhibition zone surrounding each well after incubation. Providing valuable information about the effectiveness of the tested drug against the tested bacteria strain, this zone of inhibition indicates how much the drug inhibited the growth of the bacteria. Drugs or substances are evaluated for their antimicrobial activity using the agar diffusion method or disk diffusion method.

Inoculum preparations

- Bacterial inoculum

Inoculums were prepared by inoculating Staphylococcus aureus, Escherichia coli, and Bacillus subtilis into nutrient broth. After 24 hours of incubation, the broth inoculated with bacteria was allowed to cool to room temperature. A nutrient-rich environment of the broth fostered the growth of bacteria within the inoculums during incubation. For subsequent use in the experiment, they reached sufficient concentrations and metabolic activities. Using the agar diffusion method previously described, the resulting bacteria cultures were then considered inoculums and ready for further experiments or testing.

Agar cup plate method

A pressure of 15 lb/in^2 and a temperature of 121°C were used to sterilize the culture medium during an autoclaving process. Aseptically transferred sterilized glass Petri dish plates were used to transfer the sterilized medium. In order to form a gel-like surface, these plates were left at room temperature for several hours. A Petri dish plate was subsequently inoculated with 15g of bacteria inoculum. The solidified medium was carefully drilled into four wells with diameters of 6mm each using a sterile borer. A sterile micropipette was used to add different

concentrations of drugs to each well. In order to allow the drugs to diffuse into the surrounding medium, the plates were kept undisturbed for two hours. A 24-hour incubation period was conducted with the Petri dishes inverted after the diffusion period. As a result of this incubation period, bacteria were able to grow and interact with the drug samples. In an effort to measure the diameter of the zone of inhibition surrounding each well, a measurement was taken after 24 hours. Using the zone of inhibition, we can determine how effectively the drug inhibited bacterial growth, indicating its antibacterial effectiveness. In this way, the antimicrobial properties of the drugs can be evaluated by determining how susceptible bacteria are to different concentrations of the drugs. [11] [12]

Determination of *Invitro* Anti-Oxidant Activity

Free radical scavenging method (H_2O_2)

Instrument used: UV-Visible spectrophotometer

Reagents used: H_2O_2 and 0.1M Phosphate buffer- pH 7.4

Procedure

Test solutions of different concentrations were prepared as part of the procedure. One ml of each test solution was diluted with 3.8 ml of 0.1 M phosphate buffer solution. A hydrogen peroxide solution of 0.2 ml was then added to the mixture. After incubating for 10 minutes, the reaction mixture was analysed at 230 nm for absorbance. In addition to the blank reaction mixture without the sample, a blank sample without the reagents was also prepared. Standardization was performed using ascorbic acid. In order to calculate hydrogen peroxide inhibition percentage, the following formula was used:

Percentage inhibition = [(Absorbance of control - Absorbance of sample) / Absorbance of control] x 100

An absorbance value at 230 nm indicates the optical density of the reaction mixture. Table 6 and Fig 5 show the readings. **[13-16]**

Results and Discussion

Table: 1 Macroscopy of *Klenia grandiflora*

S. No	Characteristics	Results observed
1.	Colour	Whitish Green
2.	Odour	Characteristic odour
3.	Taste	Characteristic taste
4.	Shape	Elliptic

Fig:1 Habitat of *Klenia grandiflora*

Dorsal surface Ventral surface

Fig:2 Macroscopy of *Klenia grandiflora*

Microscopy

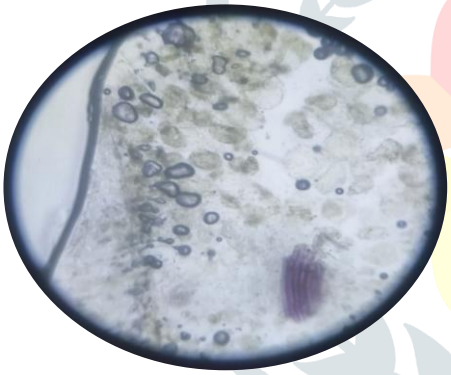

Fig: 3 Transverse section of leaves of *Klenia grandiflora*

Table- 2: Physico-chemical constant determinants of *Klenia grandiflora*

S. No	Physico-chemical constants	*Percentage yield of K. grandiflora*
1	Foreign matter	1.5%w/w
2	Loss on drying	5.76% w/w
3	Extractive value- Alcoholic extract	8.92% w/w

4	Extractive value- Water extractive	6.65% w/w
5	Ash value	8.2% w/w

Table 3: Determination of physical parameters of leaves of *Klenia grandiflora*

S.No	Physical parameter	*K. grandiflora*
1	Final yield value	12.25%w/w
2	Consistency	Semi solid
3	Color	Green

Table-4: Preliminary Phytochemical screening of HAEKG

S.No	PHYTOCHEMICAL	*K. grandiflora*
1)	Anthraquinone glycosides	+
2)	Alkaloids	+
3)	Phenolic compounds	+
4)	Flavonoid's	+
5)	Saponins	-
6)	Steroids	-

(+) Presence (-) Absence

Anti-bacterial activity

The Crude extracts of *Klenia grandiflora*. The leaf extracts gave the highest yield of 12.2 %w/w, they are different concentrations were screened for their anti-bacterial activity against *S. aureus, B. substilis & E. coli* as representations for Gram-positive & Gram-negative bacteria. Respectively, Gentamicin at a concentration of 10µg/ml was used as a positive control.

Table:5 Diameter of inhibition of zone of grow thin inhibition of HAEKG against different bacterial strains using the agar cup plate method

Samples	Diameter of inhibition zone(mm)		
	S. aureus	B. substilis	E. coli
HAEKG (10µg/ml)	2.2 ± 0.2	2.3 ± 0.2	2.0 ± 0.2
HAEKG (20µg/ml)	2.6 ± 0.3	2.4 ± 0.3	1.9 ± 0.1
Gentamicin	2.0 ± 0.2	2.2 ± 0.2	1.7 ± 0.1

Data are presented as means ± S.D (n=3)

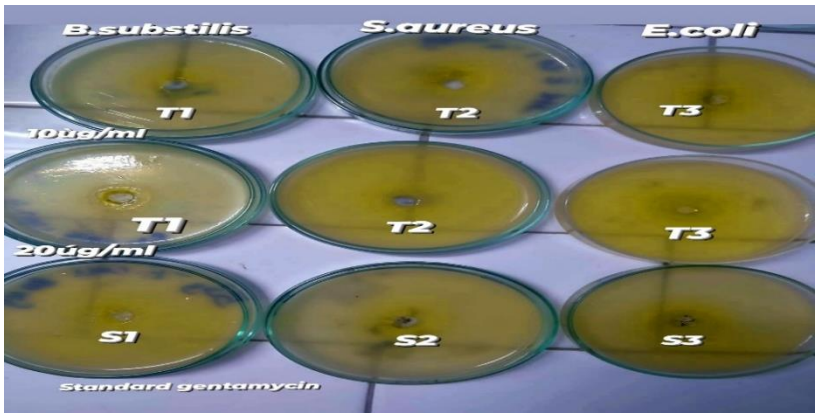

Fig-4: Diameter of inhibition zone of grow thin inhibition of HAEKG against different bacterial strains using the agar cup plate method

Invitro-Anti-oxidant activity

Studies demonstrating hydrogen peroxide scavenging activity are represented in in vitro antioxidant studies

Table:6 Determination of hydrogen peroxide scavenging activity of *Klenia grandiflora*

S.No	Concentration (µg/ml)	% of Ascorbic acid inhibition	% of HAEKG Inhibition
1	10	42.72±0.68	52.65±0.34
2	20	71.08± 0.89	75.83±1.14
3	30	82.70± 0.96	86.90±0.95
4	40	85.45± 0.86	91.01±0.83
5	50	92.42± 0.65	97.23±0.82
	IC_{50}	74.87 µg/ml	80.73 µg/ml

Data are presented as means ± S.D (n=3)

In comparison to ascorbic acid, *Klenia grandiflora* inhibits hydrogen peroxide scavenging by 80.73 g/ml

compared to 80.73 g/ml for *Klenia grandiflora*.

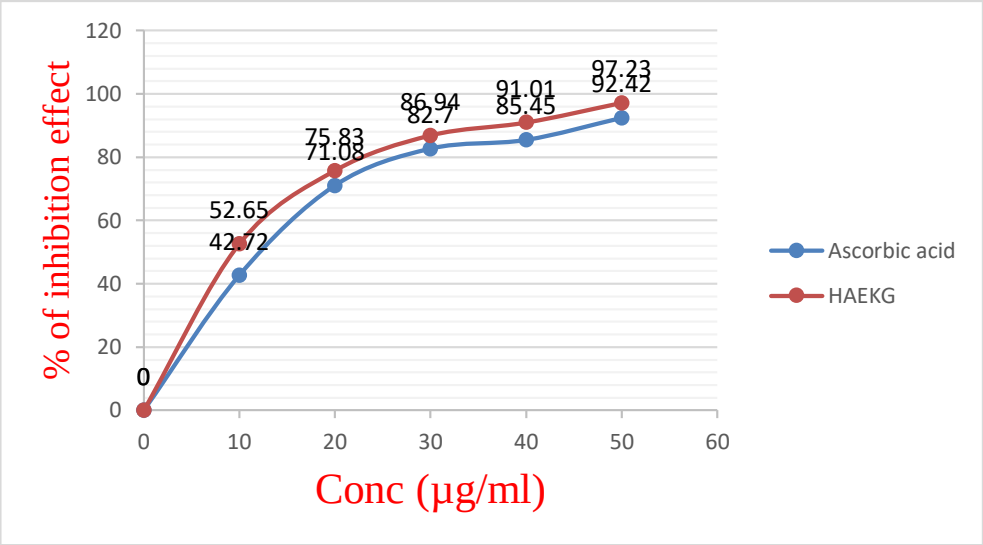

Fig:5 Determination of anti-oxidant activity of *Klenia grandiflora* by using Hydrogen peroxide scavenging effect

Conclusion

Phytochemical analysis of *Klenia grandiflora* was the focus of the current research, contributing valuable scientific insight. This plant preparation could be further formulated, according to the findings of the study. Adjuvant therapies for disease management may be possible with this formulation. This study suggests that the bioactive constituents of the plant may have therapeutic benefits and can be harnessed to develop new treatment methods. The study concludes that *Klenia grandiflora* has great potential as an alternative resource for healthcare development.

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