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Phytochemical Screening of Aqueous Leaf, Stem Bark and Root Extracts of *Rhaphiolepis bibas* (Lour.)

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Abstract:- Various plant extracts or their bioactive components are used in traditional medicine. Phytochemicals found in medicinal plants play a key role in the traditional treatment of several diseases. Despite advances in modern medicine, plant-based remedies are increasingly sorted due to their lesser side effects. This study's goal was to examine the presence of phytochemical compounds in the aqueous root, stem bark, and leaf extracts of *Rhaphiolepis bibas* gathered from Kiambu county, Kenya. The presence of various phytochemicals such as alkaloids, saponins, glycosides, terpenoids, steroids, phenols, tannins, and flavonoids has been confirmed by qualitative phytochemical analysis of these plant aqueous root, stem bark, and leaf extracts.

Keywords: *Secondary Metabolites; Flavonoids; Tannins;Antioxidant.*

I. INTRODUCTION

Since the beginning of recorded human history, humans have employed medicinal plants to treat a variety of ailments, demonstrating their significance in the drug discovery process (Fransworth, 2008). Researchers have always been guided by the use of traditional folk remedies made from wild plants to find new treatments for promoting healthy living in both people and animals (Achterberg, 2013). However, there are still other therapeutic herbs hidden within the plant that needs to be evaluated scientifically.

Plants produce an array of bioactive molecules which makes them a rich resource for folk role medicine, nutraceuticals, food supplements, drug manufacturing industries, and chemical entities for synthetic drug formulation (Pollio *et al.*, 2016; Cutillo *et al.*, 2004). The arousal of the worldwide interest in ethnopharmacology reflects recognition of the plant's potent antioxidant activities, no or minimal side effects, and economic viability as well as their availability in our ecosystem (Azmathullah *et al.*, 2011).

Phytochemicals with antioxidant activities protect the body against free radicals because they slow down or stop the oxidation of vital biomolecules such as lipids, proteins, carbohydrates, and nucleic acids, preventing the initiation and propagation of the reaction and delaying the oxidation process. As a result, natural antioxidant sources and human use of various substances with antioxidant activity are of great interest. Phenols, flavonoids, vitamin C, and carotenoids are the most prominent antioxidants (Arika *et al.*, 2019).

This study involves the determination of the phytochemical profile of aqueous stem bark, leaf, and root extracts of *Rhaphiolepis bibas.* This plant is a subtropical, substantially big evergreen tree of the Rosacea family Maloideae, a family whose origin is in China where it has been grown for more than 2,000 years. More than 30 countries in the world including Japan, India, and Mediterranean countries in Europe, Australia, Madagascar, New Zealand, Kenya, and South Africa introduced the plant to the farmers, but its commercial cultivation is only done in a few countries (Rajalakshmi *et al.*, 2017). Its medicinal and dietary usefulness was determined by the identification of some of the active chemicals in the makeup of these plants part. The medicinal and nutritional value of these plants makes them valuable in the medical and therapeutic fields.

Fig 1 Rhaphiolepis bibas

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II. MATERIALS AND METHODS

A. Collection of Plant Material

Fresh leaves, stem barks, and roots of *R. bibas* were collected from Kiambu County, Gatundu Sub-county, at latitudes-0.910711; longitudes 36.78334 at altitude 2127 m above Sea level. The plant materials were taxonomically identified by a taxonomist at the National Museums and a voucher specimen (I.W.K-001) was kept for future reference. The fresh stem bark, roots, and leaves were washed clean with tap water and rinsed with distilled water, and then shade-dried with occasional turning to achieve uniformity in drying. The roots and stem barks were first chopped into small pieces and shade dried for one month. When the plant materials were completely dry, they were crushed into powder with a mechanical mill. The powdered materials were packed in properly labeled khaki bags and stored at room temperature (25 ± 1 ºC).

B. Preparation of Plant Extracts

One hundred grams of the dried samples were separately soaked in a liter of distilled water. They were maintained at 60 ºC in a water bath and left to stand for 24 hours, after which the extracts were first decanted and the remainder squeezed through a muslin cloth. The extracts were vacuum filtered and concentrated through lyophilization. The percentage yield of each crude extract was then calculated using a formula described by Marie (Helmenstine,2020).

% Extract yield = (mass of crude extract obtained)/(mass of soaked powdered material) ×100.

C. Qualitative Phytochemical Screening of the Extracts

R. bibas lyophilized extracts were screened for phytochemicals which are the different secondary metabolites associated with antibacterial activities. For the detection of various plant constituents, standard modified protocols described by Joshi *et al.*, (2010) were used.

- *Test for Alkaloids*

In different test tubes, around 0.1 g of the aqueous root, stem bark and leaf extracts of *R.bibas* was mixed with 10% H_2SO_4 and boiled slightly in a water bath for 3 minutes before filtering. A cream hue was seen after mixing the filtrate (1ml) with Mayer's reagent (two drops). Dilute ammonia was used to alkalinize the rest of the filtrate after which 2 ml of Chloroform was added for filtrate separation. The remaining chloroform was evaporated which left a hard residue. To the residues, 10% H_2SO_4 (0.2 ml) was added and put into two equal portions. A drop of Wagner's reagent was then added. The presence of alkaloids or nitrogen-containing compounds was indicated by the appearance of an orange precipitate.

- *Froth Test for Saponins*

To 2 ml of *R. bibas* aqueous leaf, stem bark, and root extract 1.0 g of Sodium Bicarbonate was put. After vortexing, the mixture was held at 25ºC for fifteen minutes undisturbed. The formation of a stable froth was observed as a positive test for saponins.

- *Salkowski Test for Steroids*

One g of lyophilized material was soaked in 10 ml of pure Chloroform in a test tube. This was then warmed for 5 minutes in a water bath at 60°C. The aqueous extracts Chloroform combination was filtered and cooled while still hot. 2 ml filtrate was collected, and 2 ml H_2O_2 was added. Steroids were indicated by a yellow ring that appears at the interphase and turns red after 2 minutes.

- *Test for Flavonoids*

Ten mls of 70% ethanol were added to 1 g of each extract, which was then boiled for 5 minutes. While still hot, the extracts were filtered and then chilled. After that, for each ethanol extract, a filter paper was soaked and then reacted with ammonia gas. Flavonoids' presence was demonstrated when a yellow zone on the filter paper was formed. A bright yellow precipitate was formed with the addition of 2 mls of dilute Sodium Hydroxide indicating flavonoids.

- *Keller-Kalian Test for Glycosides*

Glycosides are substances that, when hydrolyzed, yield one or more sugars (glycones) and a non-sugar molecule (aglycone or genine). Three drops of Ferric Chloride and concentrated Sulphuric acid were mixed with the extract solution in glacial acetic acid. Observation of a reddish-brown tinge at the interphase of the two layers with a bluish-green hue in the upper region indicates a positive test for deoxy sugars.

- *Test for Terpenoids*

To 4 mg of the leaf, root and stem bark of *R. bibas* ground material Acetic Anhydride and Chloroform 0.5 mls each were added in a test tube and mixed. To the mixture, concentrated Sulphuric acid solution was slowly added. The observation of a red-violet color confirmed the presence of terpenoids while a greenish-bluish coloration further confirmed the steroid's presence.

- *Test for Phenols*

For 5 minutes 1 g of the root, stem bark, and leaf extract were boiled in 10 mls of 10% Ethanol. After filtering, the hot extract was chilled. Two milliliters of individual extract were then mixed with one milliliter of Ferric Chloride. A greenish precipitate indicates a positive test for phenols.

- *Test for Tannins*

In a 25 mls conical flask, 1 gram of the powdered extract was dispersed in 10 mls of distilled H_2O. This was boiled gently for 10 minutes. With the help of muslin cloth, the samples were filtered. 2 mls of the filtrate were placed in a test tube to which Ferric Chloride was added dropwise. Gallic tannins had a blue color, while catechol tannins had a green-black color.

- *Test for Quinones*

After weighing 1 g of the lyophilized aqueous crude extract, it was dissolved in 3 mls of H_2O_2 before boiling for 5 minutes. While still hot, filtration was done and the filtrate cooled in ice. 3 mls of Carbon Tetrachloride were added to the filtrate and mixed. The carbon tetrachloride fraction was separated in a separating funnel. 2 drops of ammonia were

added with vortexing. Anthraquinones were confirmed when the ammonical layer was examined for a rose pink to crimson color.

➢ *Test for Fixed Oils.*

To 0.5 g of the plant material, 5 mls Petroleum Ether was added. The extract was subjected to vaporization by pouring the mixture onto a filter paper in the open air for a few minutes. The presence of fixed oils was shown by the presence of translucency on filter paper.

D. Data Management

For each screening procedure, the test reagent was introduced to the prepared sample and a color change was observed. According to the results of the test, the result was reported as either present (+) or absent (-). All experiments were done in triplicates.

III. RESULTS AND DISCUSSION

➢ *Qualitative Phytochemical Screening of Aqueous Extracts of E. japonica*

The presence of various phytochemicals in *R. bibas* aqueous root, leaf, and stem bark extracts was investigated in the current study. The results showed that alkaloids, terpenoids, saponins, quinones, tannins, glycosides, steroids, and fixed oils were present in root and stem bark extracts as shown in Table 1. The aqueous leaf extract tested negative for steroids and fixed oils.

Table 1. Qualitative Phytochemical composition of aqueous extracts of *R. bibas*

Key: + present; - absent

Phytochemical	Root extract	Stem bark extract	Leaves extract
Saponins	+	+	+
Tannins	+	+	+
Glycosides	+	+	+
Phenols	+	+	+
Flavonoids	+	+	+
Quinones	+	+	+
Terpenoids	+	+	+
Alkaloid	+	+	+
Steroids	+	+	-
Fixed Oils	+	+	-

➢ *Discussion*

The presence of phytochemicals such as phenols, tannins, flavonoids, saponins, steroids, terpenoids, and alkaloids, which are known to exhibit physiological as well as therapeutic properties, was discovered through analysis of the plant extracts. Alkaloids have been found to have analgesic, antispasmodic, and antimicrobial effects (Saranraj & Sivasakthi, 2014; Okwu and Okwu,2004). Several studies have shown that glycosides can reduce blood pressure (Han *et al.*, 2007). Apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection, enhancement of endothelial function, suppression of angiogenesis, and cell proliferation activities are just a few of the biological features of phenolic compounds (Shrestha *et al.*, 2015).

Moreover, saponins, which are known to have anti-inflammatory effects, were found in the plant extracts. In addition to being very essential substances because of their interactions with other substances like sex hormones, steroids have been discovered to have antibacterial characteristics. (Epand *et al.*, 2007; Okwu, 2001). Moreover, the aqueous *R. bibas* extracts showed the presence of tannins which have been demonstrated to have antioxidant activity (Kaczmarek *et al.*, 2020). As well, tannins can prevent the growth of a wide variety of fungi, yeasts, bacteria, and viruses. Tannins and terpenoids are responsible for folk role medicine's analgesic and anti-inflammatory effects. The physiological and biosynthetic processes occurring inside the plant at different parts during growth can be reasonably linked to the absence of some phytochemicals in one sample and their presence in the other.

Antioxidants directly counteract the effects of free radicals by neutralizing, interacting, or competing with a substrate containing molecular oxygen as its terminal electron acceptor (O_2). As a result, molecular oxygen serves as a thermodynamic sink. Natural antioxidants obtained from medicinal plants are a superior alternative to oxidative damage because synthetic antioxidants are considered harmful and carcinogenic. Herbal medicines can be utilized to cure and prevent degenerative disorders brought by oxidative stress (Arika *et al.*, 2019). Since plant phytochemicals have diverse polarities, different solvents extract antioxidant molecules differently. The yield, as well as the ROS scavenging power of a plant extract, depends mainly on the extraction solvent used (Nabavi *et al.*, 2008).

Alkaloids were reported to have *in vitro* antioxidant properties. In addition, the oils present in *R. bibas* aqueous extracts might as well have contributed to the free radical capture potency of the extract. Ismail *et al*. 2010, showed that fatty oils have DPPH radical scavenging activity. Saponins have detergent-like features and Arabski *et al*., 2012, found that crude saponin extracts of *A. indicum* leaves have promising antibacterial and antioxidant properties.

Therefore, it is important and useful to conduct preliminary phytochemical screening to identify chemical components in plant material that could lead to a quantitative estimation of those components as well as to identify the source of chemical compounds that are pharmacologically active(Sharanabasappa *et al*., 2007). The study's findings so imply that the detected phytochemical compounds may be the bioactive components, and these plants are demonstrating their value as a source of bioactive substances with significant medical value.

IV. CONCLUSION

The findings showed that *R. bibas* aqueous extracts possess components with significant therapeutic value. There are many pieces of evidence from past investigations that support the bioactivity of the detected phytochemicals. Many

studies have shown that the presence of these phytochemicals gives plants physiological and therapeutic qualities that can be used to treat a variety of diseases. As a result, *R. bibas* aqueous extracts may be a reliable source for therapeutic medicines. It is strongly advised that these plants be used in traditional medicine, and it is also proposed that more research be done to identify, purify, and isolate the medicinal ingredients that give this plant the observed effects. Also, more research is encouraged to determine whether this plant has the claimed health benefits and determine the possible mode of action.

DECLARATIONS

➢ *Author Contribution Statement*

Ibrahim Waweru Kariuki: Performed the experiments; Analyzed and interpreted the data; Contributed reagents and materials, Wrote the paper.

Mathew Piero Ngugi: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials,

John Maingi: Contributed reagents and materials.

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➢ *Competing Interest Statement*

The authors declare no conflict of interest.

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