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Phytochemical Screening and Medicinal Properties of Ethanolic Leaf Extract of *Harungana madagascariensis*

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ABSTRACT

Common in Madagascar, Mauritius, and some other Tropical African nations is the herbal remedy Harungana madagascariensis. It is frequently observed at low elevations in evergreen woods, along river banks, and along forest margins. It is also known as the "Dragon Blood Tree." The Harungana madagascariensis plant is a member of the genius, which consists of a single species from the Hypericaceae family. Its been employed as a medicinal herb in the conventional treatment of many illnesses, including anemia, piles, kidney disorders, trypanosomiasis, dysentery, and malaria. Generally speaking, various phytochemical components of herbs are responsible for their therapeutic activities, particularly their antibacterial potentials. The major phytochemical components of the ethanolic Harungana madagascariensis leaf extract were examined in the current study. This can help healthcare professionals of alternative therapies comprehend the pharmacology and pharmacognosy of the plant, and most crucially, decide how the herb should be delivered. Findings confirmed that the herb's therapeutic efficacy is due to the presence of flavonoids, saponin, glycosides, and other compounds.

Keywords: *phytochemical constituents, flavonoids, glycosides, alkaloids, medicinal properties.*

INTRODUCTION

Herbal remedies are those whose leaves, stems, barks, or roots are at least partially employed for medical reasons [1]. They are documented to include compounds that are biologically significant or serve as building blocks for the manufacture of effective medicines [2]. The value of medicinal plants to the wellbeing of people and communities has been acknowledged over time. According to claims, these medicinal plants contain biologically active substances like sugars, proteins, enzymes, fats and oils, minerals, nutrients, alkaloids, anthraquinones, terpenes, flavonoids, anthocyanins, carotenoids, sterols, simple phenolic glycosides, tannins, saponins, and polyphenols, to name a few. These substances have medicinal activities [3] that are either therapeutically significant or are precursors. These herbs are the first option for rural residents who have restricted access to conventional healthcare because they are readily available and almost completely free of charge compared to orthodox pharmaceuticals. [4,5]. One of these conventionally used medicines for treating anemia is *harungana madagascariensis* [6]. Dragon blood tree is one of its popular names. The *Harungana madagascariensis* plant is a member of the genius, which consists of a single variety from the Hypericaceae family.

H. madagascariensis and medicinal potential

H. madagascariensis is specifically utilized to treat disorders that display blood, including skin issues. The leaves are employed as a medicine for bleeding, diarrhea, gonorrhea, sore throats, headaches, and fevers, whereas the sap is used to treat scabies and as

an anthelmintic (worm) [7]. While the liquid that the inner peel has indeed been cooked in is utilized as a therapy for dysentery and is also given to babies who are constipated and windy, the sap produced from the inner bark is taken gently warmed as a purgative [8]. Tapeworm remedies have been made from sap rinsed out of bark removed from the east and west sides of the tree trunk, and the back is also utilized as a vermifuge [8]. The resinous sap is applied externally to treat all manners of cutaneous complaints including leprosy, sores, itch, scabies, ringworm, craw-craw, etc **[8].**

Another treatment for diarrhea, bleeding piles, trypanosomosis, fever, cold, cough, malaria, and jaundice is the infusion of plant roots and stem bark [7]. While the roots and bark were boiled in water and the infusion is consumed twice daily to disrupt the menses, the roots are also used to promote breast development in young women [7]. Treatment for suspected liver or kidney problems uses the plant's aqueous crude extract.

Aphrodisiac, astringent, oxytocic, nauseous, emmenagogue, expectorant, hemostatic, purgative, styptic, and vermifuge properties are believed to exist in the bark, sap, and gum, which are highly prized [8]. Cuts, especially ulcers and recently healed circumcised incisions, are treated with gum. Additionally, the dry gum is used to cover wounds.

Comparable to the peel and sap, the branches, leaflets, and leaf sprouts are also used medicinally to cure a variety of ailments. The leaves and flowers are occasionally used as an asthma treatment [8]. The bark and leaves are used for stomach problems in various parts of Africa. Additionally regarded as febrifugal and anti-malarial, the stems and flowers. They have also been applied to the management of heart issues [8]. The fruits are sometimes used during abortions since it is thought that the red juice stops bleeding. The fruit is diuretic, stomachic, and, when consumed in excessive amounts, emetic [9].

Toxicity

According to World Health (WHO) studies, 70–80% of the globe's populations rely on complementary and alternative medicine, primarily herbal sources, for their primary care [10]. Additionally, false assumptions that these medications have no side effects have been made [11]. Although medicinal herbs may have a variety of biological effects in people, relatively little is known regarding their safety in overall, and this is also true of *H. madagascariensis*. Toxicological testing is done on a variety of experimental animals to forecast the toxicity as well as provide recommendations for choosing a "safe" dose in humans in order to ascertain the suitability of medications and plant products destined for human use [12].

MATERIAL AND METHODS

Preparation of ethanolic leaf extract of *H. madagascariensis*

The extract was made using a technique that was reported in [13]. 1000g of *H. madagascariensis* leaves extract were soaked in 2500ml of 98% pure ethanol solution, stirred, and allowed to stand for 24 hours. The solution was then funneled into a beaker after being filtered with white cotton fabric and a Whatman filter paper. The volume was then dumped into a stainless steel tray and dried using a centrifugal evaporator.

Then, 108.43g of the *Harungana madagascariensis* ethanol fresh leaves was scraped into a simple sample paper plate using a spatula. By serially diluting of an extract, a stock solution was created from the yields at the appropriate doses. Until the time of treatment, the dilute extract was kept at room temperature.

Phytochemical analysis

Phytochemical testing was done on the ethanol extract of leaves of the *Harungana madagascariensis* plant. 20 cc of pure water was used to dissolve 2g of the crude extract. Using established techniques, the solvent was tested for alkaloids, flavonoids, tannins, saponins, terpenes, arthroquinones, and glycosides [14,15].

Test for alkaloids

Mayer's test: Two test jars were filled with 2 ml each of the supernatant and control solution. 3 drops of Mayer's reagents were applied to the test tubes. After thoroughly combining the liquids, they were let to stand for 5 minutes before being checked for residue and color alterations.

Wagner's test: Two test tubes were filled with 2 ml each of the filtrate and control solutions. Wagner's reagent was applied in three drops to the test tubes. After thoroughly combining the treatments, they

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were let to stand for 5 minutes before being checked for residue and color alterations.

Dragendorf's test: Two test tubes were filled with two milliliters each of the filtrate and control solutions. Three drops were added to the test tubes. After thoroughly combining the treatments, they were let to stand for 5 minutes before being checked for residue and color alterations. [13, 15,16].

Test for tannins

Different testing tubes each contained 2 ml of the filtrate and control solution. There were three drops of 10% ferric chloride applied to the test tubes. We looked at the combinations to check for sediment and color changes.

Into two different test tubes, 2 ml each of the filtrate and control solution were pipetted. Three drops of 10% lead acetate were applied to each test tube. The combinations were tested for precipitate formation and color alterations [16,17].

Test for flavonoids

The supernatant plus control treatments were subsequently pipetted into two different test tubes with a volume of about 2 ml each. Three drops of NaOH were applied to the test tubes. After letting the combinations rest for two minutes, they were checked for residue and color changes.

The filtrate and control solutions were pipetted into two different test tubes, each holding 2 ml. Three drops of NaOH and three drops of 0.5 N HCl were added to the test tubes. We looked at the combinations for precipitation and color changes. [16,17]

Test for saponnins

2 ml of the filtrate and the control solutions were poured into two different test tubes for the emulsification test. Three drops of olive oil were added to each test tube, and the mixture was briskly agitated. The existence of brown dispersion in the combinations was checked [16,17].

1 ml of the extract and the control liquid were diluted into two different test tubes for the frothing test. 4 ml volumetric flask were applied to the test tubes. The concoction was forcefully mixed before being checked for foaming.

Test for glycoside

Different test tubes containing 2 ml of the filtrate plus control fluids as well as 2 ml of the Fehlings I and II solutions were used. After a thorough mixing process, the solutions were heated in a water bath for two minutes. The combination was examined for sedimentation and color alterations [16,17].

Test for terpenes

The powdered sample was prepared by dissolving 10 mg of pure chloroform with a mass of about 0.1 g. For this test, the filtered solution was employed.

In separate test tubes, 1 ml each of the filtrate and reference treatments received 1 ml of acetic anhydride. A glass rod was used to thoroughly combine the solutions. The test tubes were then angled, and 1 ml H2SO4 was poured into each test tube's mixture from the side. The existence of a color shift at the junction of the two liquid layers was investigated [16,17].

RESULT

Table 1: Phytochemical analysis result of *H. madagascariensis* ethanolic leaf extract

PHYTOCHEMICAL CONSTITUENT	CONCENTRATION
Alkaloids	+++
Flavonoids	+++
Saponnins	+++
Glycosides	+++
Phenolic compounds	++
Terpenes	++
Tannins	+
Steroids	-

+++ = Present in very high concentration
++ = Present in appreciable concentration
+ = present in low concentration
- = absent

DISCUSSION

Many different illnesses have been treated with the herb Harungana madagascariensis [18]. Traditional uses for the leaves and roots include the treatment of fever, anemia, nephrosis, malaria, gastro-intestinal problems, and malaria [19]. The use of such an antibacterial treatment for gastrointestinal diseases has been supported by research showing that

aqueous leaf extract exhibits anti-microbial activity against several species of bacteria, including Bacillus subtilis, Staphylococcus aureus, Eschericia coli, and Salmonella typhi [20]. At less than 10 g/ml, the aqueous extracts prevented Entamoeba hystolytica from growing. On an isolated guinea-pig ileum, the roots extract likewise showed a more than 70% inhibition of acetylcholine and potassium chloride solution-induced contractions [21]. Jubi Formula, a herbal supplement that has been shown to improve hemoglobin (Hb) content and pack cell volume (PCV) in anemic patients, contains Harungana madagascariensis as one of its active ingredients [22]. One of the plants used to treat malaria is Harungana madagascariensis, according to an ethnobotanical study of several natural remedies used by traditional healers in Zimbabwe's Mola, Kariba area, to treating and preventing malaria. However, recent research has looked at the effects of crude extracts and pure compounds from a hexane extract of the leaves of Harungana madagascariensis for their analgesic, anti-inflammatory, alpha-glucosidase inhibition, and anti-oxidant activities, respectively [25]. Earlier studies of Harungana madagascariensis were based on the stem bark [20, 23, 24]. Six identified substances from the foliage of Harungana madagascariensis were recently demonstrated to have anti-plasmodial properties in vitro [26]. In vitro tests on a variety of isolated natural product components have revealed powerful antimalarial activity [27]. In vivo studies of the same chemicals have been demonstrated to be devoid of such actions, according to studies [28].

Acute Toxicity Test

H. madagascariensis's ethanol extract leaf had an extremely high LD50 of 4699.20 mg/kg body weight. The aqueous extracts of H. madagascariensis is practically non-toxic and can be advised for human consumption due to the high value of the fatal dose (LD50) [29]. However, as changes in soil content have been discovered to have some influence on the toxicity of herbs, toxicity tests should indeed be undertaken of plants derived from diverse soils.

Phytochemical Screening

The assessment of H. madagascariensis fresh leaves for the preliminary phytochemical, flavonoids, saponins, and glycosides revealed that these compounds were present in extremely high amounts. While is in low concentration, terpenes and phenolic chemicals are also present in significant amounts. These phytoconstituents elements are found in extracts that have significant antioxidant capacity, support tissue regeneration, lessen blood capillary permeability, and boost hemolysis resistance [30]. These compounds' existence and associated qualities warrant their use in medicine.

Flavonoids

A group of polyphenolic bioactive molecules found in plants and fungi are called flavonoids (or bioflavonoids). Chemically, flavonoids consist of a heterocyclic ring, two phenyl rings (A and B), and a 15-carbon skeleton in general (C). The abbreviation for this carbon structure is C6-C3-C6. They can be divided into the following categories using the IUPAC nomenclature:

a. *flavonoids* or *bioflavonoids*

b. *isoflavonoids*, derived from 3-phenylchromen-4-one (3-phenyl-1,4-benzopyrone) structure

c. *neoflavonoids*, derived from 4-phenylcoumarine (4-phenyl-1,2-benzopyrone) structure

Anthoxanthins are ketone-containing chemicals that belong towards the three aforementioned flavonoid groups (flavones and flavonols). The word "bioflavonoids" was initially used to describe this class. Additionally, non-ketone polyhydroxy polyphenol chemicals, which are more precisely referred to as flavanoids, have been described using the names flavonoid and bioflavonoid in a more general sense. Ring A, B, and C are the three cycles or heterocycles that make up the flavonoid core. Phloroglucinol substitution mainly occurs in Ring A. Flavonoids are abundantly present in plants and serve a variety of purposes. The most significant plant colours are flavonoids, which provide yellow or red/blue coloring in flower petals intended to draw pollinating animals. Flavonoids play a role in UV filtration, symbiotic nitrogen fixation, and flower coloring in higher plants. They might also function as physiological regulators, signaling molecules, and cell cycle inhibitors [31, 32]. The plants that mostly contain flavonoids can prevent Helicobacter pylori infection and treat peptic ulcers [32].

Flavonoids have strong antioxidant properties and can protect the body from poisons. It is a strategy to support the body's ability to maintain health and may lessen the likelihood of the body developing some

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chronic health issues. Flavanols, for example, are recognized for their antioxidant qualities. Some forms of flavonoids have specialized activities. As a result, they might aid in the management of heart disease symptoms. Foods including onions, kale, grapes, red wine, tea, peaches, berries, tomatoes, lettuce, and other items are frequently found to contain flavanols. The flavan-3-ols These kinds of flavonoids are found in foods that are particularly nutrient-dense. White tea, green tea, oolong tea, black tea, apples, grapes (both purple and red), blueberries, strawberries, cocoa, and chocolate-related goods are among them.

Flavones are well recognized for their ability to reduce inflammation in the body. They are made of peppermint and red peppers.

It is well known that flavanones are effective at lowering cholesterol and body weight. These additionally possess anti-inflammatory qualities. Lemons, limes, oranges, and grapefruit all contain them. The body's hormones may be kept in balance by isoflavones. Consequently, isoflavonoids, which are mostly found in soy, soy products, and legumes like fava beans, are utilized to treat hormonal imbalance in the body. Flavonoids assist in controlling cellular activity and fend against free radicals that subject your system to peroxidation. In plainer terms, they make the body work more effectively while safeguarding it against poisons and stresses that are present every day. Additionally effective antioxidants are flavonoids. Antioxidant properties aid the body's defenses against potentially hazardous chemicals that may be ingested. One of the body's immunological reactions is inflammation. Inflammation can be brought on by allergens, bacteria, toxins, as well as other irritants, which can cause unpleasant symptoms. Flavonoids may assist the body in suppressing that inflammatory response, so reducing the symptoms [32].

Consuming a lot of flavonoids may help lower your chances of developing type 2 diabetes. According to Trusted Source's 2018 meta-analysis, a high consumption of dietary flavonoids is associated with a decreased risk of type 2 diabetes. To demonstrate the effectiveness of flavonoids as blood sugar controllers, more study is necessary. Additionally, flavonoids have demonstrated antioxidant and anti-inflammatory properties. Certain flavonoids may aid in preventing the growth of cancer cells, according to research. Maintaining a balanced diet and consuming foods high in flavonoids may reduce your risk of developing specific cancers [32].

Saponins

Chemicals called saponins are found in a variety of herbs, seeds, and vegetables. They are also present in sea creatures and starfish. They are utilized in vaccine formulations in medicine to control immune response. These substances are included in shampoos, soap, household cleansers, and cosmetics due to their antibacterial and foamy qualities [16].
The advantages of saponins for health have been confirmed by several studies throughout the years. These substances could lower cholesterol levels, eliminate disease-causing microorganisms, remove oxidative stress, and stop the growth of tumors. They enhance lipid metabolism, which may help prevent and treat obesity, according to recent studies [16].
Saponins help to maintain cardiovascular health by reducing blood cholesterol and body fat. According to researchers, these compounds prevent the absorption of cholesterol. Clinical studies have demonstrated that the saponins in ginseng and ginger lower total and LDL (bad) cholesterol without affecting HDL (good) cholesterol levels [16].

Hyperlipidemia is a significant risk factor for atherosclerosis, chronic renal disease, heart disease, and other illnesses. This chemical can cause artery blockages, which can result in plaque formation on the blood vessel walls. The heart consequently receives less blood and oxygen. A diet high in saponins may aid in preventing these issues and keeping a healthy heart.

Because saponins possess hypoglycemic qualities that maintain blood sugar levels within normal ranges and avoid insulin surges, they may have a positive impact on body weight. They also prevent the enzyme lipase, which breaks down dietary fats, from being released into the environment. The body will consequently absorb less fat from food [16].

In laboratory tests, mice fed saponins had lower levels of cholesterol, triglycerides, and belly fat. The favorable effects of saponins on body weight are thought to be further enhanced by the possibility that they prevent the development of adipose tissue and reduce hunger.

Saponins have an antibacterial quality that defends the body from viruses, germs, and fungi. Additionally, they enhance immunological performance by promoting T-cell generation. They also serve as antioxidants and neutralize oxidative stress. These

substances are utilized in several vaccines as a result. These plant compounds have traditionally been investigated for their potential to enhance immune response and trigger cancer cell death. They also combat free radical harm, which increases the likelihood of developing cancer. These substances have been demonstrated in laboratory experiments to kill leukemia cells and prevent their spread all through the body [16].

Glycosides

According to the aglycone's chemical characteristics, glycosides are likewise divided into several categories. This taxonomy is the most helpful in terms of biochemistry and pharmacology [33, 34, 35, 36, 37, 38, 39, 40].

Alcoholic glycosides: Salicin, a member of the genus Salix, is an illustration of an alcoholic glycoside. Salicylic acid, which is produced in the body and is chemically similar to aspirin, is generated from salicin and possesses analgesic, antipyretic, and anti-inflammatory properties.

An aglycone group, a derivative of anthraquinone, is present in anthraquinone glycosides. They act as a laxative. Excluding the monocots of the family Liliaceae, they are primarily found in dicot plants. Senna, rhubarb, and Aloe species all contain them. Anthraquinone is converted to anthron and anthranol.

Coumarin or a precursor is the aglycone in coumarin glycosides. Apterin is one example, which has been shown to both widen and inhibit calcium channels in the blood vessels. Psorale acorylifolia's dried leaves can be used to make more coumarin glycosides. Chromone glycosides: The aglycone in this instance is known as benzo-gamma-pyrone. The aglycone in this case is a flavonoid glycoside. This vast class of glycosides includes, for instance:

- Hesperin (aglycone: hesperetin, glycone: rutinose)

The Naringin (aglycone: naringenin, glycone: rutinose)

- Rutin (aglycone: quercetin, glycone: rutinose)
- Querectin (aglycone: quercetin, glycone: rhamnose)

The antioxidant properties of flavonoids are among their significant effects. Additionally, they are believed to lessen capillary fragility.

Phenolic glycosides: The aglycone in this case is a straightforward phenolic structure. Arbutin, which is present in the Common Bearberry Arctostaphylosuva-ursi, is one example. It acts as a urogenital antiseptic.

Phenolic Compounds

In nature, natural antioxidants are a common type of phytochemical. This means that these substances must be obtained mostly from food and medicinal herbs because they cannot be produced by the human body. Plant extracts rich in phenolic compounds have gained popularity in recent years as a way to improve the quality of food. Numerous research have also been based on their medicinal usage as active constituents. However, there is still a lot of work to be done. The findings presented in this special issue provide fresh perspectives on how processing influences the phenolic composition of foods and the bioactivity of phenolics in nano-encapsulated form on cancer cells. Polyphenols can be found naturally in olives and olive oil. Their phenolic components' and antioxidant properties' impacts on heath are strongly related. However, in order to produce tasty goods, the table olive sector needs to reduce the bitterness of olive phenolic compounds. In this instance, fresh technologies that seek to address environmental sustainability issues have been developed together with altered processing techniques, such as acid, base, and/or enzymatic hydrolysis. In a different setting, siltation, storage, and centrifugation can all affect the polyphenol content and other minor constituents in olive oil [41].

It has been examined and connected to the caliber of olive oil. Dietary polyphenols can be found in significant amounts in cereals, particularly whole grains. This special issue provides a foundation for supporting the growth and use of cereals by summarizing how physicochemical processing may affect the phenolic composition of grains. On two blackberry cultivars, the impact of various organic fertilization techniques, as well as harvest date and storage period, has been assessed in the case of blackberry fruits [41].

It has been demonstrated that the fruits' various physicochemical and antioxidant qualities could perhaps offer various shelf-lives when they are sold to the public as new products. Bioactive chemicals have been effectively encapsulated and delivered to desired sites using nanoparticle distribution channels. This

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special issue contains a study on nanoencapsulated phenolics from Callistemon citrinus extract, berberine, and a combination of both that increased their bioactivity against three tumor cell lines by almost 2-fold. We are happy to introduce this special issue, which contains the above studies with intriguing findings about the interaction between both the three variables of food production, phenolic compounds, and attractiveness [41].

Alkaloids

Alkaloids are naturally occurring chemicals with nitrogen that have a variety of pharmacological effects, such as anti-malarial, anti-asthmatic, anti-cancer, cholinomimetic, vasodilatory, anti-arrhythmic, analgesic, antibacterial, and anti-hyperglycemic ones. Alkaloids also have stimulant and psychoactive effects.

CONCLUSION

The study emphasizes the effectiveness of ethnomedicinal plants, which have been utilized for centuries in various regions of India. This ancient idea should be thoroughly examined in the context of current medical knowledge and, if appropriate, might be partially applied.

The Harungana madagascariensis leaf extracts includes a variety of phytochemicals, including a cardiac glycoside, phenolic compounds, flavonoids, and alkaloids, according to a phytochemical investigation. The leaf extract shows strong antioxidant properties, according to the quantitative DPPH assay. This explains why it is used medicinally.

Consent And Ethical Approval

As per university standard guideline, participant consent and ethical approval have been collected and preserved by the authors

Competing interests

Authors have declared that no competing interests exist.

Authors' Contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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