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Antihemolytic activity of herbal drugs

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Abstract
Red blood cell rupturing, as well as inheriting genetic defects and an immunological condition, all lead to haemolysis and, eventually, illnesses like thalassemia and sickle cell anaemia. Medicinal medicines' antihaemolytic action can cancel out their impact. Foods high in antioxidants have a significant role in illness prevention. Our research aimed to identify herbal herbs that efficiently cure hemolytic anaemia. Based on their traditional usage and literature, these plants were chosen for their antihaemolytic properties. Four plants known for their antioxidant qualities were employed in this study (Solanum virginianum, Morus Alba, Psidium guajava, Hyssopus officinalis). The concentrations of extracts that decreased haemolysis by 50% (IC50 values) for S. amplexicaules, R. nasutus, and R. tetraphylla extracts were 142, 157, and 135 ug/ml, respectively. The extraction technique and extract concentration were both important in preventing hemolysis. These plants might provide easily available natural antioxidants to the pharmaceutical industry.

Keywords: antihemolytic, antioxidant, solanum virginianum, morus alba, psidium guajava, hyssopus officinalis, S. amplexicaules, R. nasutus, R. tetraphylla

Introduction

Haemolysis

Haemolysis is the breakdown (lysis) of red blood cells (erythrocytes) and release of their components (cytoplasm) into the surrounding fluid (e.g., plasma). Haemolytic anaemia is a blood condition that happens when your red blood cells break down or die quicker than your body can replace them with new blood cells. Haemolytic anaemia can be caused by inheriting anaemia-causing genetic disorders, some infections, and certain drugs. [1]

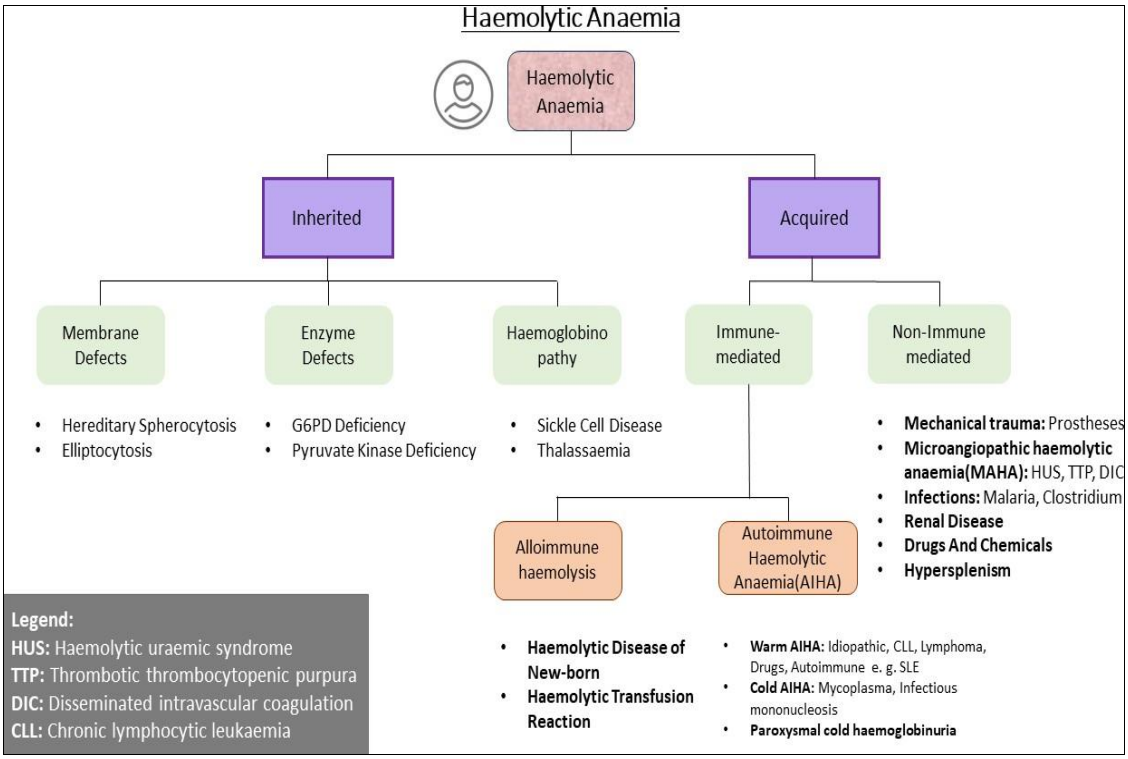

Fig 1: Haemolytic anaemia classification

Table 1: Drugs that can cause haemolytic anaemia

Cephalosporins	Methyldopa
Dapsone	Nitrofurantoin
Levodopa	Nonsteroidal anti-inflammatory drugs
Levofloxacin	Penicillin and its derivatives

Significance Of Antihemolytic Drugs/Treatments

AIHA is an immunological condition caused on by antibodies that are directed towards unmodified autologous red cells [2] It results from the production of specific complement- and/or non-complement-activating

autoantibodies to red blood cells (RBCs). These activating autoantibodies may lead to RBC destruction of variable extent (haemolysis) and anaemia [3]. The disorder may be a primary (idiopathic) or a secondary disease [2]. The autoantibodies may be warm-reactive with a temperature optimum at 37 °C or cold-reactive with a temperature optimum way below the normal body temperature [4]. The autoantibodies may be warm-reactive with a temperature optimum at 37 °C or cold-reactive with a temperature optimum way below the normal body temperature [4]. Cold antibodies are less frequently seen, but warm antibodies are the main cause of AIHA. Cold antibody AIHA are mainly secondary anaemia, whereas half of warm antibody-based AIHA are idiopathic anaemia [2].

At the moment, all available treatments are based on short retrospective studies, case reports, suggestions, conventional methods, or self-empirical experiences. Additionally, every medication used to treat AIHA targets the patient's natural immune system in some way, which could cause the immune system to be severely compromised as a result of the medication.

In warm antibody AIHA, the standard first-line treatment consists of glucocorticosteroids with or without high-dose immunoglobulins, while splenectomy is considered a second-line treatment. Among other immunosuppressive treatments, rituximab (anti-CD20) appears to be very effective in patients with AIHA hot antibodies resistant to standard therapy. In AIHA patients with underlying autoimmune or lymphoproliferative disease, mycophenolate mofetil is extremely beneficial [2].

Steroids and splenectomy are ineffective for treating patients with cold antibody AIHA. Although responses to rituximab are typically short, half of these patients may respond to it [2]. Sometimes, following effective anti-lymphoma or anti-tumour therapy, AIHA that is linked to malignant lymphomas or tumours resolves [2].

Warm AIHA treatment

The majority of instances of AIHA are caused by warm-reacting autoantibodies [6]. About 50% of warm AIHA cases are classified as primary or idiopathic, with the remaining cases developing in response to an underlying malignancy, an immunological disorder, an infectious condition, or a medication trigger. Idiopathic warm AIHA typically affects people in their fourth or fifth decade, with females being more frequently affected than males [7].

Typically, immunosuppressive medications, splenectomy, and corticosteroids are used to treat AIHA. A few novel treatments have become available in recent years, and some of them have shown some success. Patients who are not candidates for splenectomy or who do not respond to it, patients who relapse after splenectomy, and patients who cannot maintain stable haemoglobin levels without using intolerably large doses of corticosteroids are the key populations for this therapy.

First line treatment

Corticosteroids are generally accepted as the first-line treatment for patients with warm antibody type AIHA, despite the fact that their usage is based more on experience than on scientific research. As per general consensus, steroids at a dose of 1 mg/kg per day of prednisone should be used as first-line treatment, with nearly 80% of patients achieving a response with corticosteroids alone [10, 8]. The objective of treatment is to raise baseline hemoglobin levels to 9 to 10 g/dL, which is often accomplished within the first three weeks of treatment. To prevent a symptomatic return, a slow taper spanning several months is frequently necessary. The majority of adults need long-term steroid therapy to maintain remission, even if response rates to steroids are high, with up to 20% needing dosages of more than 15 mg/d [8]. In addition to reducing antibody synthesis, steroids may also have direct effects on autoantibody RBC affinity and have suppressive effects on tissue macrophage phagocytosis, which could explain why they are effective in treating warm AIHA [10].

Second line treatment

Second-line therapy is necessary for patients who do not react to steroids, need large long-term doses, or have unacceptably high adverse effects. Splenectomy and rituximab therapy are the two second-line treatments for warm AIHA that are most widely accepted. Splenectomy has a success rate of about 50%, but to establish a long-lasting remission, continued low-dose steroids may also be necessary [10, 12]. Rituximab, an anti-CD20 monoclonal antibody, is a second-line therapy treatment option to splenectomy. Standard dosing of four weekly infusions at 375 mg/m2, dosing for autoimmune diseases twice at 1000 mg given two weeks apart, or even a modest fixed dose of 100 mg per week for four treatments are all examples of treatment regimens [13, 5]. Initial responses are observed in a majority (80%) of patients, but relapse in 1 to 3 years is common and may necessitate repeat therapy [8][13]. First-line therapy with concurrent corticosteroid medication has also been evaluated, and it appears to produce a more lasting response than first-line therapy with corticosteroids alone [14].

Treatment options beyond second-line therapy

Immunosuppressive treatment was often recommended as preferred second-line treatment because response rates of 40% to 60% have been claimed in earlier reviews [8]. Treatment options in refractory cases include azathioprine or cyclophosphamide and other immunosuppressive drugs such as cyclosporine or mycophenolate mofetil; however, published case series on these regimens are small [9] [8]. Severe refractory disease has been successfully treated with high-dose cyclophosphamide [15].

Transfusion

The requirement for transfusion is the initial assessment for individuals who present with AIHA. True cross-matching is typically not attainable because the autoantibodies in warm AIHA frequently cause pan agglutination of donor RBCs. However, in emergency situations, transfusion is frequently successfully performed with blood that is ABO and Rh-D matched [8]. It is advised to start with a gradual infusion to prevent an abrupt transfusion reaction. Despite the fact that pan reactive autoantibodies may hemolyze a large portion of transfused blood, the temporary boost in oxygen-carrying capacity can be crucial [9, 8].

An approach to transfusion in a patient with warm AIHA includes [8].

- Blood transfusion using least incompatible or best-matched blood.

a. Due to the heated autoantibodies' pan agglutinating capabilities, full cross-matching is frequently not achievable.
b. ABO- and Rh-D-matched units in patients who are unlikely to already have alloantibodies, such as females who have never been pregnant or undergone a transfusion or males who have never undergone a transfusion.
c. In non-urgent instances or in situations where a significant risk of alloimmunization is suspected, extended phenotype matching for additional Rh subgroups (C, c, E, e, Kell, Kidd, S, s) is available.

- Test blood infusion to keep an eye on acute transfusion responses.

a. An initial blood infusion of 20 to 30 mL.
b. Watch for 20–30 minutes.
c. If there is no reaction, carry out the planned transfusion.

Drawbacks Of Antihemolytics Drugs/Treatments

Autoimmune haemolytic anaemia (AIHA) is a rare illness characterised by autoantibodies directed against self-red blood cells, with an estimated incidence of 0.8-3 per 105/year in adults, a frequency of 17:100,000, and a death rate of 11%.[16, 17] Because there is just one randomised research [18] and few prospective phase II studies, the therapy of AIHA is currently not evidence-based. [19, 20] There is no medicine that can stop or compensate for severe haemolysis. Furthermore, none of the medications used are always successful, and the positive impact is unpredictable. [41] All therapy possibilities are currently based on short retrospective research, case reports, suggestions, traditional therapies, or self-empirical experiences. [17, 24, 42, 43, 44]

Treatment of warm AIHA

First-line therapy

Corticosteroids

Corticosteroids are widely accepted as the first-line therapy for individuals with warm antibody type AIHA. In fact, there is little published data on their efficacy, [16, 21, 22] Clinical trials do not support this. [23] Corticosteroids, often prednisone, are administered at an initial dosage of 1.0-1.5 mg/kg/day for 1-3 weeks, or until hemoglobin levels greater than 10 g/dL are achieved. [23] Response occurs mostly during the second week, and if no or minor improvement is shown in the third week, this therapy is considered useless. [23] First-line corticosteroid therapy is likely to give a response in 70- 85% of patients; however, only one in three instances remains in long-term remission once the medicine is withdrawn, another 50% requires maintenance doses, and roughly 20-30% need subsequent second-line therapies. It is unknown how many adult patients are healed just with steroids; however, it is thought that less than 20% of patients are cured. [17] Patients who do not respond to first-line treatment should be evaluated for a suspected underlying illness, since AIHA linked with malignant tumors, ulcerative colitis, benign ovarian teratomas, or IgM warm autoantibodies is frequently steroid-refractory. [17] Many individuals with comorbidities, such as diabetes and uncontrolled hypertension, obesity, osteoporosis, peptic ulcer, psychosis, cataract, and preterm, are substantially or completely contraindicated to steroids (children). Furthermore, all of these comorbidities may be caused by steroid medication. [41] Side effects are substantially more common in people who have been using steroids for a long time, and have also been noticed in many patients who only take 5-7.5 mg per day. Cushing's syndrome is thus not an uncommon consequence in such circumstances. [41]

Second-line therapy

Second-line treatments include splenectomy and rituximab. [17]

Splenectomy

One disadvantage of splenectomy is the absence of accurate predictors of prognosis, as its effectiveness is unrelated to disease duration, responsiveness to steroids, or the amount of splenic sequestration. [24] Furthermore, Despite the fact that laparoscopic intervention reduces surgical risk (0.5-1.6% vs. 6%), splenectomy may be linked with surgical consequences (pulmonary embolism, intra-abdominal hemorrhage, abdominal abscess, abdominal wall hematoma). [25] Thrombosis induced by encapsulated bacteria is the most feared complication following splenectomy, with a risk of 3.3-5% and a death rate of up to 50%. [25, 26, 41] Even with pre-operative pneumococcal, meningococcal, and Hemophilus immunization, the usefulness and efficiency of antibiotic prophylaxis in this scenario is questionable, and not all studies suggest it. [16, 28] Finally, thromboembolism and pulmonary hypertension are small but considerable concerns. [29, 30] It is unknown how common splenectomy occurs in adults. [17] In a large pediatrics series of 256 AIHA patients, splenectomy was performed in 13.9% of cases (99 of whom had Evans syndrome).[31] should be noted that, despite the fact that the incidence of infection in children and adults is reported to be similar, children have a higher death rate (1.7% vs. 1.3%). [26]

Rituximab

Rituximab therapy is well tolerated, with the majority of patients seeing no adverse events other than infusion-related side effects. [32, 33, 34] Although there have been reports of rare occurrences of progressive multifocal encephalopathy, especially in onco- hematologic diseases, hepatitis B reactivation, and other viral infections, the medicine has a well-established safety profile (infectious events occur in roughly 7% of patients). [35, 36] Antiviral prophylaxis is now suggested to avoid reactivation of hepatitis B following rituximab and extended steroid treatment. [37] In addition, there is evidence that rituximab can produce serious adverse effects such as infections, serum sickness, acute respiratory distress, and perhaps the development of malignant lymphoma. [45, 46, 47, 48, 49]

Third-line therapy

Immunosuppressive drugs

All patients tolerated the treatment of azathioprine (100-150 mg/day), cyclophosphamide (100 mg/day), and cyclosporine A well. [23] Abdominal pain, significant leukopenia, a persistent increase in liver enzymes, or other severe side effects are signs of azathioprine intolerance. [41] The most common side effects of cyclophosphamide are leukopenia, infections, impaired fertility, teratogenicity, and an increase in the prevalence of neoplasia. [16] There is limited data on the use of mycophenolate mofetil in place of azathioprine in patients with refractory warm AIHA. [23]

Fourth line therapy

Due to the high toxicity of high-dose cyclophosphamide and alemtuzumab, it is considered a "last resort" option in severe idiopathic AIHA that has not responded to all previous treatments. [17]

Other options

Danazol combined prednisone showed a great response as first-line therapy (8 of 10 patients), but it was less effective (3 of 7) in relapsed or refractory patients. [38]

a. In AIHA, intravenous immunoglobulins (IVIG) are routinely utilized, either alone or in conjunction with prednisone. [39] A recent AIHA recommendation said that high-dose immunoglobulin should only be used in life-threatening situations. [40]
b. Plasma exchange was used as a stopgap measure in a limited number of critically ill warm AIHA patients, including children and adults, whose anemia could not be controlled with steroids and transfusion treatment alone. [16]

Hematopoietic stem cell transplantation

1. Sanz *et al* [51] documented 12 patients who developed severe AIHA (eight with cold antibodies and four with warm antibodies) after a median of 147 days (range, 41–170). Between 1996 and 2004, 272 adult patients with various malignant hematopoietic diseases had stem cell transplants. The only independent variables linked with AIHA, according to multivariate analysis, were HSCT from unrelated donors (P=0.02) and the development of chronic severe GVHD (P=0.018). [50]
2. Wang *et al* [52] reported 19 occurrences of AIHA (overall incidence, 3.6%) among 533 allogeneic HSCT patients at King's College Hospital in London in 2015. The median period from HSCT to AIHA was 202 days, and in three instances (adenovirus, Cytomegalovirus (CMV), and pneumonia of unknown cause), AIHA was preceded by an infective episode. [50]
3. Between 2000 and 2015, major Spanish joint research in adults and children identified 60 occurrences of AIHA among 4099 allo-HSCT patients (cumulative incidence 1.5%). AIHA appeared 6 months after the transplant on average, and it was related with HLA mismatch donor (P=0.005), cord blood usage (P=0.005), and age 15 years (P=0.005). [53, 50]

Supportive therapy (RBC transfusion)

To maintain clinically acceptable haemoglobin levels, AIHA patients may require RBC infusions. [23] Undiagnosed alloantibodies might be the source of increased haemolysis following transfusion, which could be misinterpreted as a worsening of AIHA severity. [16] Not only should the volume of blood transfused be kept to a minimum, but RBCs should be given slowly, preferably at a rate of no more than 1 mL/kg/h. [16]

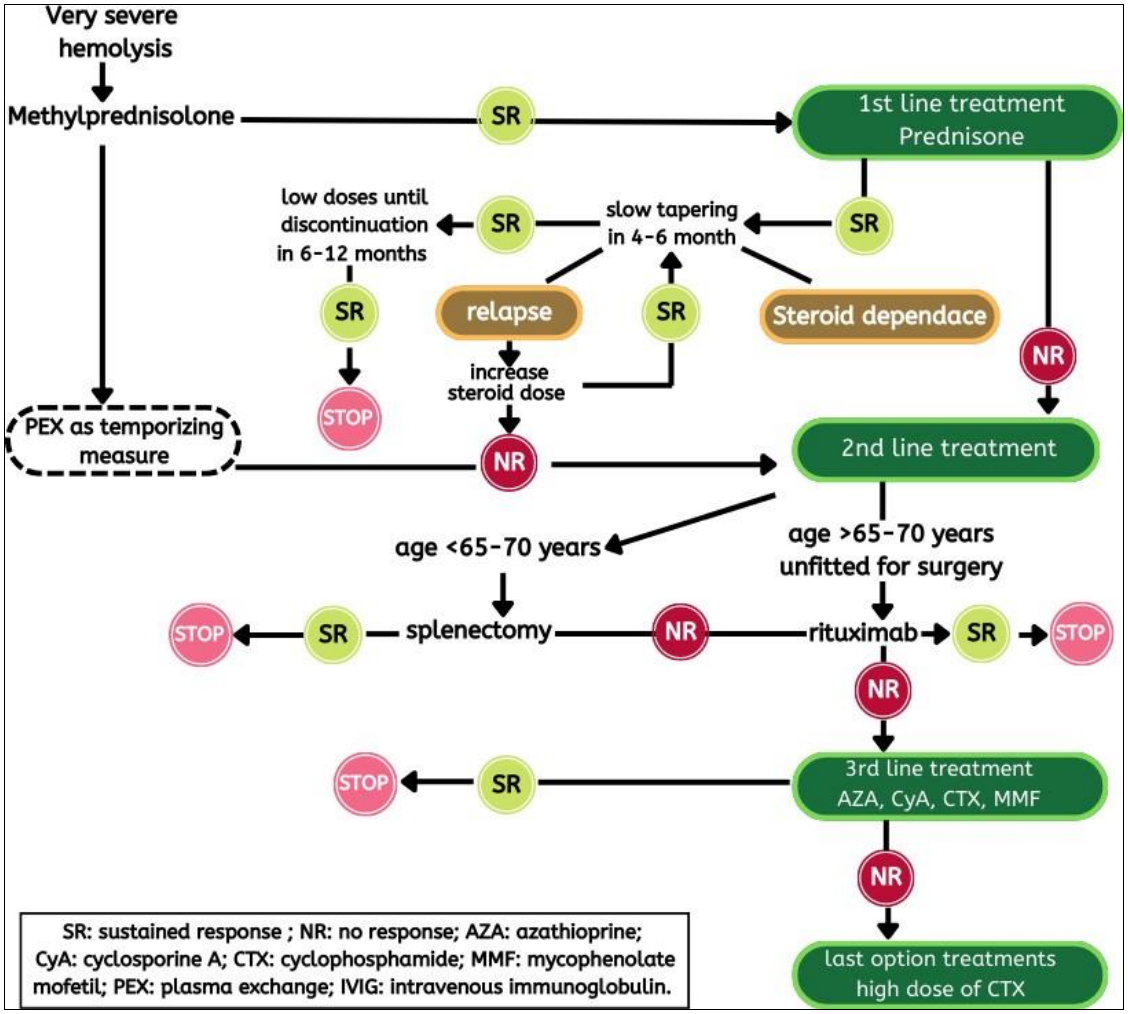

Fig 2: Treatment of severe haemolysis

Herbal Plants with Antihemolytic Activity

Solanum Virginianum

Indians mostly use the medicinal herb *Solanum virginianum*, also referred as Surattense nightshade, yellow-fruit nightshade, yellow-berry nightshade, Thai green eggplant, or Thai striped eggplant (from the unripe fruit). The fruit, for instance, is a poisonous portion of the plant. Common names for the plant comprise Kantakari, Solanum surattense Brum.f and Solanum xanthocarpum. Other names were Indian night shade and yellow berried night shade plant. *Solanum virginianum L.* is equivalent with Schrad and Wendl.

Fig 3: Solanum virginianum

S. virginianum L. is a member of the Solanaceae family, also referenced to as yellow berried nightshade. Recent research has highlighted the potential application of *S. virginianum* in contemporary medicine. Red blood cells (erythrocytes) burst (lyse) and release their cytoplasm into the fluid surrounding them during hemolysis (e.g., blood plasma). The antihemolytic formula and spectrophotometric method are employed to determine the antihemolytic activity of the plant extract. Materials and Procedures the study focuses on the S. virginianum ethanol extract's antihemolytic properties. A positive patient's erythrocytes are used to create a suspension, and an *in vitro* technique is used to perform a haemolytic activity. The extract's antihemolytic potential was evaluated using a spectrophotometric technique, validated by infusing the plant, and matched to the norm. The formula beneath was applied to quantify the extracts' percentage level of hemolysis: At- An/Ac-Anx100 = % of hemolysis.

Results

Based on the report's findings, ethanol extracts from the *S. virginianum* plant are seldom neither poisonous nor hazardous to human erythrocytes. The test tube with the greatest concentration of the plant extract had a hemolytic effect, whereas the test tube with the minimum concentration of the plant extract had an antihemolytic impact. The experiment significantly and dose-dependently enhanced the hemolytic activity of the S. virginianum plant extract.

Hyssopus Officialness

Hyssopus officinalis, popularly known as hyssop, is a shrub which is indigenous to Southern Europe, the Middle East, and the region around the Caspian Sea. It is a member of the Lamiaceae, or mint family. It has been adopted in conventional herbal medicine owing to its purported abilities as an expectorant, antiseptic, and cough suppressant.

Description

A shrub or subshrub with vivid colors, hyssop blooms somewhere around 30 and 60 cm (12 to 24 in) high. A number of erect branches emerge from the woody base of the stem. The plant bears lance-shaped, 2.5 cm (34 to 1 in) long, dark green leaves. Hyssop blooms throughout the summer amid fragrant pink, blue, or, lesser occasionally, white flowers. These generate tiny, oblong tetra-achenes.

Fig 4: Hyssopus officinalis

From the Veresk region (in the middle Elburz Mountains), plant material aerial components of *Hyssopus angustifolius M. Bieb* have indeed been recovered. Iran until the spring of 2010 (latitude: 35° 54' N, longitude: 52° 59' E, altitude: 1900 m). The plant was authenticated as legitimate at the University of Mazandaran's Herbarium of Biology (voucher specimen No 975) [65]

Mulberry

White mulberry, common mulberry, and silkworm mulberry are all synonyms for the *Morus Alba* species inside the Moraceae family. The leaves on robust new branches can attain a maximum length of 30 cm (12 in), are deeply lobed, thin, and have rounded lobes. Generally, 5-15 cm (2.0-5.9 in) long, lobed, cord-shaped at the base and rounded to the apex, and serrated at the margins, the leaves of older plants have a rounded to elliptical form. Trees are usually deciduous in temperate climates, while tropical vegetation can be evergreen. Although they can develop on the same tree, male and female blossoms often are found on independent trees. 1-1.5 cm (0.4-0.6 in) is really how long the fruit is. Dark purple in color in the wild, it could range from white to pink in different crops. Unlike the stronger flavors of red and blackberries, it is sweet yet bland. Fruit-eating birds' droppings contain the seeds, which are widely spread. Scientifically, the white mulberry is renowned for its quick plant movement linked to the discharge of pollen from

its cucurbit. For about 25 s, stamens may rocket accumulated elastic energy into the air. [66]

Mulberry fruits are an excellent source of polyphenol, notably anthocyanins, which have significant outcomes such as minimizing the risk of cancer and cardiovascular disease because of their anti-inflammatory, antioxidant, and chemoprotective characteristics. [67] The mulberry (*Morus Alba*), a deciduous, swift-growing, medium-sized tree, with a 1,000- year history of usage in sericulture. Nearly every continent in the globe has mulberry trees

Fig 5: Mulberry

as natives [68]. Because it can survive high levels of air pollution, it is appreciated for landscaping, urban gardening, street shade, and city decoration. In Chinese medicine, the whole mulberry plant, including the leaves, fruits, bark, and branches, has been utilized for centuries. Today, mulberries are utilized to create a wide range of goods, including jams, juices, wine, vinegar, ice cream, marmalade, and other food-related items. The mulberry fruit is used by individuals as a treatment to protect liver damage, strengthen joints, lowering blood pressure, and smooth bowel movements, and treat early hair greying, weakness, weariness, laxative, and anemia. [69, 70, 71]

The functional quality, delectable flavor, and nutritional significance of mulberries have resulted in a dramatic rise in production and consumption in recent years. [72] Anthocyanin, a category of naturally occurring polyphenolic chemicals that give mulberry fruit extracts their color, are present in significant concentrations. Due to its affordable price, high polyphenol content, and natural -glycosidase inhibitor concentration, mulberry fruit is currently competitive on the market when compared to other berries. Here, we isolated anthocyanin from mulberry fruit and examined the antioxidant and anti-hemolytic effects to better understand the anthocyanin-rich extract and its bioactivity. [67]

Psidium guajava

Common guavas sometimes referred as yellow guavas, lemon guavas, or apple guavas, are tiny, evergreen trees or shrubs that are indigenous to the Caribbean, Central America, and South America. It is easily pollinated by insects; when grown, the common honey bee, *Apis mellifera*, is the primary pollinator.

Fig 6: Psidium guajava

Numerous chemicals have allegedly been discovered in guava leaves. Numerous components, such as lipids, carbohydrates, proteins, vitamin C, essential oils, tannins, saponins, triterpene acids, and polyphenols have indeed been detected in the phytochemical analysis of the ethanolic extract of guava leaves [54, 55, 56]. Gallic acid, quercetin, protocatechuic acid, chlorogenic acid, caffeic acid, kaempferol, ferulic acid, morin, and quercetin-3-o-glucopyranoside were listed as polyphenols in ethanolic extract of guava leaves [54, 57]. Comparative research on the antioxidant activity of quercetin, morin, and quercetin-3-o-glucopyranoside revealed quercetin to be the most active, while quercetin and morin were combined to provide synergistic antioxidant activity [56]. In light of this, utilizing crude extract could provide significant antioxidant activity.

Anti-hemolytic properties the extracts' potential to inhibit ferrous ion-induced hemolysis was established. We systematically investigated the extracts' influence on human RBCs and concluded that they had no negative impacts (data not shown). At small concentrations, all extracts exhibited sufficient haemolysis inhibitory effects. At a concentration of 3.125 g/ml, the PG extract had a maximal anti-haemolytic activity of 91.5 0.99%. At a concentration of 50 g/ml, PO and RN had maximum anti-haemolytic activity of 83.25 3.66 and 77.88 6.13%, respectively. The PG extract outperformed the PO and RN extracts in terms of effectiveness among these three extracts, in that order. In this article, the antioxidant activity of three Thai medicinal plants was assessed using the DPPH radical scavenging test, the ABTS radical decolorization assay, and the iron-binding assay. The most used spectrophotometric technique is the DPPH and ABTS test because it is straightforward, quick, sensitive, and repeatable. By donating an atom of hydrogen to the DPPH radical and creating a non-radical molecule, the DPPH radical scavenging test was utilised to assess the antiradical potential of extracts [58]. ABTS radicals are more reactive than DPPH. An electron transfer process takes place during the reaction. It has been widely employed to test the antioxidant potential to bleach the ABTS cation [59]. We discovered that although not to the same extent, the PG, PO, and RN extracts have the ability to scavenge DPPH and ABTS radicals. With an IC50 value of 6.25 g/ml for DPPH and 2.75 g/ml for the ABTS radical scavenging test, the PG extract demonstrated a notable antioxidant action. This outcome was consistent with the findings of Chen and his colleagues [60], who evaluated the antioxidant activity of four aqueous extracts of nutraceutical herbs, including P.

guajava, Camellia sinensis, Toona sinensis, and Rosmarinus officinalis, and discovered that P. guajava leaves extract had the highest level of activity. [87]

Antihaemolytic activity of H2O2-induced RBCs-

Peroxyl radicals produced by H2O2 also inhibited the hemolysis of cow RBCs in a concentration-dependent manner. S. amplexicaules, R. nasutus, and R. tetraphylla extracts had IC50 values of 142, 157, and 135 ug/ml, respectively, at which they reduced hemolysis by 50%. The standard's IC50 value for BHT was 91 g/ml. Tocopherols, isoflavones, and phenols of various structural types [61] all inhibit the H2O2-induced hemolysis. Another way that phenolic compounds prevent hemolysis may be through their particular interactions with membrane phospholipids and proteins, which result in membrane stabilization and decreased permeability [63].

Table 2: IC50 values of the methanol extracts in antioxidant properties of medicinal plants.

Sample	Antihaemolytic activity (µg extract/ml)	ABTS (µg extract/ml)	DPPH (µg extract/ml)	Reducing capacity (µg extract/ml)
BHT	91 ± 1.57	45 ± 0.64	70 ± 0.37	165 ± 0.15
R. tetraphylla	135 ± 1.12	80 ± 1.22	170 ± 0.46	200 ± 0.11
R. nasutus	157 ± 1.06	93 ± 1.51	230 ± 1.83	>250
S. amplexicaulis	142 ± 1.33	120 ± 1.36	190 ± 1.15	>250

(n=3), ± standard deviation. IC50 value: the effective concentration at which the inhibition of radicals by 50%; reducing power was the absorbance at 0.5. [8]

Conclusion

The natural antioxidant components of plant extracts will contribute in the development of innovative drug options for antioxidant therapy. Extracts of Solanum virginianum, Morus Alba, Psidium guajava, and Hyssopus officinalis were discovered to have DPPH and ABTS radical scavenging action, albeit not at the same level. The extracts have substantial antioxidant and anti-haemolytic activities, which may be related to their high phenol and flavonoid content, according to our findings. It speaks well for future phytopharmacological research, which will investigate other biological effects or improve the mechanism of existing benefits. As a result, more research into the definitive mechanisms of Psidium guajava, Solanum virginianum, chemotherapeutic actions, and potential *in vivo* effects is warranted.

References

1. Baldwin C, Pandey J, Olarewaju O. Hemolytic Anemia. [Updated 2022 Jul 25]. In: Stat Pearls [Internet]. Treasure Island (FL): Stat Pearls Publishing, 2022.
2. Valent P, Lechner K. Diagnosis and treatment of autoimmune haemolytic anaemias in adults: a clinical review. Wiener klinische Wochenschrift,2008:120(5-6):136-151. https://doi.org/10.1007/s00508-008-0945-1
3. Salama A. Treatment Options for Primary Autoimmune Haemolytic Anaemia: A Short Comprehensive Review. Transfusion medicine and hemotherapy: offizielles Organ der Deutschen Gesellschaft fur Transfusionsmedizin und Immunhamatologie,2015:42(5):294-301. https://doi.org/10.1159/000438731
4. Berentsen S, Tjønnfjord GE. Diagnosis and treatment of cold agglutinin mediated autoimmune hemolytic anemia. Blood reviews,2012:26(3):107-115. https://doi.org/10.1016/j.blre.2012.01.002
5. Zanella A, Barcellini W. Treatment of autoimmune hemolytic anemia. Haematologica,2014:99(10):1547-1554. https://doi.org/10.3324/haematol.2014.114561
6. Sokol RJ, Hewitt S, Stamps BK. Autoimmune haemolysis: an 18-year study of 865 cases referred to a regional transfusion centre. Br Med J (Clin Res Ed),1981:282:2023
7. Gehrs BC, Friedberg RC. Autoimmune hemolytic anemia. Am J Hematol,2002:69(4):258-71. doi: 10.1002/ajh.10062. PMID: 11921020.
8. Lechner K, Jager U. How I treat autoimmune hemolytic anemia in adults. Blood,2010:116:1831-8.
9. Packman CH. Hemolytic anemia due to warm autoantibodies. Blood Rev,2008:22(1):17-31. doi: 10.1016/j.blre.2007.08.001. Epub 2007 Sep 27. PMID: 17904259
10. King KE, Ness PM. Treatment of autoimmune hemolytic anemia. Semin Hematol,2005:42(3):131-6. doi: 10.1053/j.seminhematol.2005.04.003. PMID: 16041662.
11. Barcellini W, Fattizzo B, Zaninoni A, Radice T, Nichele I, Di Bona E, *et al*. Clinical heterogeneity and predictors of outcome in primary autoimmune hemolytic anemia: a GIMEMA study of 308 patients. Blood,2014:124(19):2930-6. doi: 10.1182/blood-2014-06-583021. Epub 2014 Sep 16. PMID: 25232059.
12. Chertkow G, Dacie JV. Results of Splenectomy in Auto-Immune Haemolytic Anaemia. British Journal of Haematology,1956:2:237-249.
13. Roumier M, Loustau V, Guillaud C, *et al*. Characteristics and outcome of warm autoimmune hemolytic anemia in adults: new insights based on a single-center experience with 60 patients. Am J Hematol,2014:89:E150-5.
14. Birgens H, Frederiksen H, Hasselbalch HC, *et al*. A phase III randomized trial comparing glucocorticoid monotherapy versus glucocorticoid and rituximab in patients with autoimmune haemolytic anaemia. Br J Haematol,2013:163:393-9.
15. Moyo VM, Smith D, Brodsky I, Crilley P, Jones RJ, Brodsky RA. High-dose cyclophosphamide for refractory autoimmune hemolytic anemia. Blood,2002:100(2):704-6. doi: 10.1182/blood-2002-01-0087. PMID: 12091370. Petz LD, Garratty G. *Immune Hemolytic Anemias*. 2nd ed Philadelphia: Churchill Livingstone; 2004.
16. Lechner K, Jager U. How I treat autoimmune hemolytic anemias in adults. Blood,2010:16:1831-8.
17. Birgens H, Frederiksen H, Hasselbalch HC, Rasmussen IH, Nielsen OJ, Kjeldsen L, *et al*. A phase III randomized trial comparing glucocorticoid monotherapy versus glucocorticoid and rituximab in patients with autoimmune haemolytic anaemia. Br J Haematol,2013:163:393-9.

18. Berentsen S, Ulvestad E, Gjertsen BT, Hjorth-Hansen H, Langholm R, Knutsen H, *et al*. Rituximab for primary chronic cold agglutinin disease: a prospective study of 37 courses of therapy in 27 patients. Blood,2004:103:2925-8.
19. Barcellini W, Zaja F, Zaninoni A, Imperiali FG, Di Bona E, Fattizzo B, *et al*. Sustained response to low-dose rituximab in idiopathic autoimmune hemolytic anemia. Eur J Haematol,2013:91:546-51.
20. Naithani R, Agrawal N, Mahapatra M, Kumar R, Pati HP, Choudhry VP. Autoimmune hemolytic anemia in children, Pediatr Hematol Oncol,2007:24:309-15.
21. Gupta V, Shukla J, Bhatia BD. Autoimmune Hemolytic Anemia. Ind J Ped,2008:75:451-4.
22. Zanella, Alberto, Wilma Barcellini. "Treatment of autoimmune hemolytic anemias." Haematologica,2014:99(10):1547-54. doi:10.3324/haematol.2014.114561
23. Crowther M, Chan YL, Garbett IK, Lim W, Vickers MA, Crowther MA. Evidence- based focused review of the treatment of idiopathic warm immune hemolytic anemia in adults. Blood,2011:118:4036-40.
24. Casaccia M, Torelli P, Squarcia S, Sormani MP, Savelli A, Troilo BM, *et al*. Laparoscopic splenectomy for hematologic diseases: a preliminary analysis performed on the Italian Registry of Laparoscopic Surgery of the Spleen (IRLSS). Surg Endosc,2006:20:1214-20.
25. Bisharat N, Omari H, Lavi I, Raz R. Risk of infection and death among post- splenectomy patients. J Infect,2001:43:182-6.
26. Davidson RN, Wall RA. Prevention and management of infections in patients without a spleen. Clin Microbiol Infect,2001:7:657-60.
27. Newland A, Provan D, Myint S. Preventing severe infection after splenectomy. BMJ,2005:331:417-8.
28. Krauth MT, Lechner K, Neugebauer EA, Pabinger I. The post-operative splenic/portal vein thrombosis after splenectomy and its prevention-an unresolved issue. Haematologica,2008:93:1227-32.
29. Pepke-Zaba J, Delcroix M, Lang I, Mayer E, Jansa P, Ambroz D, *et al*. Chronic thromboembolic pulmonary hypertension (CTEPH): results from an international prospective registry. Circulation,2011:124:1973-81.
30. Aladjidi N, Leverger G, Leblanc T, Picat MQ, Michel G, Bertrand Y, *et al*. New insights into childhood autoimmune hemolytic anemia: a French national observational study of 265 children. Haematologica,2011:96:655-63.
31. Zecca M, Nobili B, Ramenghi U, Perrotta S, Amendola G, Rosito P, *et al*. Rituximab for the treatment of refractory autoimmune hemolytic anemia in children. Blood,2003:101:3857-61.
32. Rao A, Kelly M, Musselman M, Ramadas J, Wilson D, Grossman W, *et al*. Safety, efficacy, and immune reconstitution after rituximab therapy in pediatric patients with chronic or refractory hematologic autoimmune cytopenias. Pediatr Blood Cancer,2008:50:822-5.
33. Bussone G, Ribeiro E, Dechartres A, Viallard JF, Bonnotte B, Fain O, *et al*. Efficacy and safety of rituximab in adults' warm antibody autoimmune haemolytic anemia: retrospective analysis of 27 cases. Am J Hematol,2009:84:153-7.
34. Garvey B. Rituximab in the treatment of autoimmune haematological disorders. Br J Haematol,2008:14:149-69.
35. Barcellini W, Zanella A. Rituximab therapy for autoimmune haematological diseases. Eur J Intern Med,2011:22:220-9.
36. Marzano A, Angelucci E, Andreone P, Brunetto M, Bruno R, Burra P, *et al*. Prophylaxis and treatment of hepatitis B in immunocompromised patients. Dig Liver Dis,2007:39:397-408.
37. Pignon JM, Poirson E, Rochant H. Danazol in autoimmune haemolytic anaemia. Br J Haematol,1993:83:343-5.
38. Darabi K, Abdel-Wahab O, Dzik WH. Current usage of intravenous immune globulin and the rationale behind it: the Massachusetts General Hospital data and a review of the literature. Transfusion,2006:46:741-53.
39. Anderson D, Ali K, Blanchette V, Brouwers M, Couban S, Radmoor P, *et al*. Guidelines on the use of intravenous immune globulin for hematologic conditions. Transfus Med Rev,2007:21(Suppl 1):S9-S56
40. Salama A. Treatment Options for Primary Autoimmune Hemolytic Anemia: A Short Comprehensive Review. Transfus Med Hemother,2015:42(5):294-301. doi:10.1159/000438731
41. Allgood JW, Chaplin H. Jr Idiopathic acquired autoimmune hemolytic anemia. A review of forty-seven cases treated from 1955 through 1965. Am J Med,1967:43:254-273.
42. Murphy S, LoBuglio AF. Drug therapy of autoimmune hemolytic anemia. Semin Hematol,1976:13:323-334.
43. Packman CH. Hemolytic anemia due to warm autoantibodies. Blood Rev,2008:22:17-31.
44. Dunleavy K, Tay K, Wilson WH. Rituximab-associated neutropenia. Semin Hematol,2010:47:180-186.
45. Gea-Banacloche JC. Rituximab-associated infections. Semin Hematol,2010:47:187-198.
46. Kelesidis T, Daikos G, Boumpas D, Tsiodras S. Does rituximab increase the incidence of infectious complications? A narrative review. Int J Infect Dis,2011:15:e2-e16.
47. Shetty S, Ahmed AR. Preliminary analysis of mortality associated with rituximab use in autoimmune diseases. Autoimmunity,2013:46:487-496.
48. Peuvrel L, Chiffoleau A, Quéreux G, Brocard A, Saint-Jean M, Batz A, *et al*. Melanoma and rituximab: an incidental association? Dermatology,2013:226:274-278.
49. Barcellini Wilma, *et al*. "Management of refractory autoimmune hemolytic anemia after allogeneic hematopoietic stem cell transplantation: current perspectives." Journal of blood medicine,2019:10:265-278. doi:10.2147/JBM.S190327
50. Sanz J, Arriaga F, Montesinos P, *et al*. Autoimmune hemolytic anemia following allogeneic hematopoietic stem cell transplantation in adult patients. Bone Marrow Transplant,2007:39(9):555-561. doi: 10.1038/sj.bmt.1705641