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A Brief Review of Multiple Phytochemical Activities on Blackberry Leaves

Patil Prachi[1*], Patil Divakar[2], Jain Akash[3], Shaikh Azam[4], Shaikh Sameer[5], Dr. Pawar Sunil[6]
Department of Pharmaceutics, P. S. G. V. P. M's College of Pharmacy, Shahada Dist- Nandurbar
prachipatil2002.pc@gmail.com

Abstract

Blackberry leaves are not considered functional foods, despite the fact that they also have active substances with the potential to improve health. Blackberry fruits are recognised as functional foods. Blackberry (Rubus sp.) fruit has a strong antioxidant capacity and other biological activities because it includes significant levels of anthocyanins and other phenolic compounds, primarily flavonols and ellagitannins. It is well recognized that genetics, growing environments, and maturation affect the content and quantities of phenolic compounds in blackberries. Despite what is currently known about their chemistry, specialized studies on the health advantages of blackberry phenolic components, metabolism, bioavailability, and mechanism by which they bestow health advantages is rare. Age-related neurological diseases are protected against by the phenolic compounds in blackberries. Low-density lipoprotein and liposomal oxidation can be inhibited in vitro, along with illnesses and bone loss in vivo. Extracts of blackberries have also acted as an inhibitor of tumor- promoting factors and cell signalling pathways to exhibit antimutagenic actions in vitro and in animals. Blackberry phenolic compounds' anti-obesity, anti-diabetic, anti-microbial, and anti- inflammatory effects, however, require investigation. Likewise, investigations that clarify the physiologically effective quantities of phenolic chemicals found in blackberries in vivo are required.

Keywords: Rubus fruticosus L.; antioxidant; anti-inflammatory; microbiological activity.

Introduction

A blackberry is a perennial plant in the rose family (Rosaceae), Rubus fruticosus L. It is well- known for its fruit, which has therapeutic, dietary, and aesthetic benefits. With almost 700 species, the genus Rubus is the largest in the Rosaceae family. There are 12 subgenera of rubus, some of which contain domestic species [1].Volatile compounds found in the entire blackberry fruit, juice, essence, and other blackberry products total over 250 compounds. Blackberries have a wide range of volatile chemical profiles, including esters, lactones, aldehyde, terpenoids, alcohols, and acids, phenols, norisoprenoids, furanones, ketones, and furanones. Phenolic chemicals, which are

Blackberries, are the primary active ingredients [2]. Blackberry consumption has increased recently as a result of the high phenolic content and high level of vitamin C, which are chemicals used to treat degenerative disorders [3-4]. Blackberries are therefore among the aromatic medicinal plants. Blackberries clearly differ in terms of both morphology and chemical composition due to the substantial influence of the environment [5]. Tea substitutes frequently use blackberry leaves. The phenolic makeup of blackberry raw material is what essentially determines its advantages. The main bioactive components in tea are believed to be phenolic compounds [6]. Blackberry (Rubus sp.) commercial production is projected to be around 154,578 tonnes per year [7]. The primary areas for producing blackberries are North America, Europe, Asia, South America, Oceania, Central America, and Africa (in descending order of tonnes cultivated) [8]. Significant volumes of wild blackberries are also grown, which in some areas may hurt the net sales of commercially grown fruit [8].

It is nutritious and has good therapeutic properties. The plant's fruit, leaves, and stem show good potential against typical infections [9]. For diarrhoea, coughing, and fever, uses leaves. Additionally, they have a reputation for being diuretic and carminative[10]. Diarrhoea can be treated with root bark decoction [11]. Blackberries contain a natural substance called cyanidin- 3-glucoside, which has been shown in studies to have both chemo preventive and chemotherapeutic effects [12]. Fruit that has ripened is ingested to aid digestion and manage stomachaches [13]. Eye disorders can be treated with ripened fruit and Achyranthes aspera leaves when consumed together [14]. Fruits are consumed and used to make jams and jellies [10].

History / Origin

Rubus fruticosus is the origin of Rubus fruticosus is said to be in Armenia. It is now widely available throughout Asia, Europe, Oceania, North America, and South America. Blackberry is the meaning of the Latin-derived generic name Rubus. The Latin term frutico, which implies bushy or shrubby, is also the source of the word fruticosus. The origins of the blackberry are shrouded in myth. Following the Ice Age, Rubus species provided food and healing or medicinal plants for the local population. Aeschylus mentioned blackberries in his writings. In the New World, between 500 and 400 B.C.E., Hippocrates and archaeologists found evidence of Rubus species being a source of food in Newberry Crater in Bend, Oregon. Ancient Egyptians were aware of the blackberry but did not record its uses. On pages 82v–83r of the Greek pharmacopoeia "Juliana Anicia Codex or Vindobonensis Codex" was the first illustration of a blackberry with a description in a recorded form. There are many meanings associated with this plant in the religious, mythical, and ethnic spheres. These have been shown as a symbol of spiritual ignorance in Christian art. The 'Christ's crown of thorns', according to Mediterranean folklore, was constructed from Rubus fruticosus runners. The dark colour of a blackberry stands for the blood of Christ. Blackberries are also thought to be associated with unfavourable omens. The fruits of death, which are associated with Wicca and serve as a sign of sadness, were mentioned in European folklore. There is a myth found in Greek literature. As a result of trying to ride on Pegasus to Mount Olympus, a mortal named Bellerophon

slipped into a prickly shrub, was blind, and sustained other injuries. This was his cost for attempting tousurp the authority of the gods. As a result, arrogance is associated with the blackberry fruit. Hippocrates also advocated using blackberry leaves and stems that have been soaked in white wine as a treatment to speed up wound healing and ease labour pains.

Description

1. Botanical Description

Rubus fruticosus L. is a scrambling, perennial, deciduous, thorny shrub that can grow up to 3 metres at a rapid rate and is semi-prostrate to nearly upright. It grows in areas with sunny margins, dappled shade, and shady borders in woodland gardens [15]. This bushy plant has thorns, however some domesticated forms don't. Blackberries are perennial, remaining for at least three seasons [16]. Usually, plants produce semi-woody, biennial canes or stems. They range in size from slender to nearly erect, spreading bushes with thorns and leaves. The stems, which are greenish, purple, or red in colour, can grow up to 7 metres long. Each spring, the woody root's buds develop young canes that expand quickly nearly 50-80 mm per day [16] in the form of juvenile canes. In terms of branch structure, they can be divided into two groups: primocane, or vegetative cane, and floricane, or generative cane. In the second year after being produced, generative canes change from their initial vegetative state [17].

Early summer and late spring are when the plant blooms. A flower's diameter is between 2 and 3 cm, and it has five light pink or white petals. There are many stamens in flowers. Fruit forms a cluster of drupelets when the petals fall off. These drupelets start out green and eventually turn red or black as they ripen. From a business standpoint, the colour of fruit and fruit juice is crucial since people judge a product based on how it looks. The natural pigments in blackberry fruit and juice determine their colour, which in turn depends on a variety of factors, including the cultivar being studied, the agronomic practises used in cultivation, the maturity stage at which fruit is collected, the geological and climatic conditions of the area from which fruit is collected, the post-harvest storage conditions used, as well as enzymatic activity and microbial contamination. Blackberries can be frozen or fresh, and juice can be produced from both. Frozen food has considerably better colour than fresh food. Racemes or panicles of flowers and fruit are present [16]. At the tip of floricanes, they grow in groups. Blackberries bear fruit twice annually, in the spring (floricane) and the fall (primocane) [17]. The fruit is a thick cluster of discrete units, or drupelets, which become black or dark purple from red as they ripen. Round, 2-4 mm length, and medium to dark brown in hue, the seeds have irregular, deep holes. The underside of leaves are a brighter green than the upper side, which is dark green. The stalks and veins of leaves are covered in brief prickles. The base of the leaves tends to have 5 or 7 palmate leaflets, and the leaves are ternate above. These leaves have glabrous adaxial sides that are folded into pleats and dark reddish-purple in the autumn, green in the summer and deciduous in the winter [16].

2. Total Phenolic Content

Phenolic content overall of the fruits and leaves of blackberries examined. TPC was elevated in fruits as well as leaves. During the vegetation season, the TPC of leaves has increased. In most cases, there were notable variations in the harvest times. TPC was

slightly lower at the start of fall than it was in the summer, although this tendency was not statistically supported in leaves gathered in September. The TPC content of the leaves in the samples from the various growth sites was largely comparable. Nonetheless, notable distinctions were found between the leaf samples taken from cultivated and wild populations.

Fruit TPC levels were significantly greater than leaf TPC values. Overall, the study's measurement of TPC in fruits agrees well with previously released information [18-20]. According to Siriwoharn et al. (2004) [21], blackberry fruits' polyphenol and anthocyanin contents were changed at different stages of maturation. Ripe blackberries had lower TPC levels than unripe or overripe ones. Mature blackberry fruits were harvested three times during the ripening period in the current study; however, TPC did not change across the three growth sites or the sampling times. Still, notable distinctions were found between the wild and farmed fruits. Incomparison to the fruits harvested from wild blackberry plants, the cultivar "Thornfree" had a significantly higher total phenolic content in samples taken from every growth location. In contrast, Milivojevic et al. (2011) [22] found that TPC levels in samples produced in the wild were much greater, but TPC levels in both wild and farmed blackberries were abnormally low. Yilmaz et al. (2009) [23] discovered that TPC varied significantly between cultivars and a subset of wild genotypes, with the majority of the wild blackberries having higher values. However, the differences between the genotypes grown in the wild and those that were cultivated were less than those identified in the current study. Upon examining the mean values of matched wild and farmed populations for a certain location and sampling period, noteworthy positive correlations were discovered for the TPC in both leaves ($r=0.8114$, $p<0.01$) and fruits ($r=0.7624$, $p<0.05$).

$$DPPH_{\text{scavenging activity}}\ (\%) = \frac{A_0 - A_1}{A_0} \times 100\%$$

Phytochemical Analysis

1. Antioxidant Activity Assay with 2, 2-Diphenyl-1-picrylhydrazyl (DPPH)

25 µL of extracts and 175 µL of DPPH solution (3.9 mg/50 ml methanol) were mixed together. After shaking the reaction mixture, it was let to sit at room temperature in the dark for half an hour. The absorbance of 175 µL of methanol and 25 µL of water or a 3:7 v/v water- methanol mixture was measured at 517 nm in comparison to a blank, which also included 25 µL of water and a 3:7 v/v water-methanol mixture and 175 µL of methanol [24]. The analysis was conducted with six replicates. Using the following formula, the percentage of DPPH scavenging activity was calculated: Where A0 is the absorbance of the control, and A1 is the absorbance of the sample. The IC50 value, which corresponds to the concentration of the extract required to block radical production by 50%, was calculated from the results.

Oxygen Radical Absorbance Capacity (ORAC) Assay:

A sample of 10 µL was tested by combining with 170 µL of fluorescein (0.00020941 mg fluorescein/10 mL, 75 mM phosphate buffer, pH 7.4) and incubated 20 min at 37 °C. Then, 20 µL AAPH (0.14248 mg AAPH/mL buffer) was added and the fluorescence was read (excitation at 485 nm and emission at 520 nm) at the start and after 1 min, with continual shaking during the whole reaction,

until stability. The blank sample contained phosphate buffer, instead of the sample. In addition, the background from the samples was measured (a mixture containing the studied sample and DDI water only). The activity was determined using a 50 μM stock solution and 12 dilutions to obtain the Trolox equivalents [25].

$$\%\ inhibition\ activity = \frac{T_S - T_C}{T_H - T_C} \times 100\%$$

Cupric Ion Reducing Antioxidant Capacity (CUPRAC) Assay:

For the CUPRAC experiment, 50 L of extracts were combined with 150 L of CUPRAC reagent (equivalent amounts of 7.5 mM neocuproine solution in 96% ethanol, acetate buffer (pH = 7.0), and 10 mM CuCl2H2O solution) in a well of a 96-well plate [7]. The plate was shaken for five minutes before being incubated at room temperature in the dark for twenty- five minutes. At 450 nm, the absorbance was then determined. The analysis was performed on six different occasions. The IC0.5, or the concentration of extract required to produce an absorbance value of 0.5, was used to express the results.

2. Anti-Inflammatory Activity

Anti-Hyaluronidase Activity

By using a turbidimetric technique that was developed by Studzi'nska-Sroka et al. [26], the hyaluronidase inhibition was ascertained. A mixture of 25 mL of enzyme (30 U/mL in 50 mM acetate buffer pH 7.0 with 77 mM NaCl and 1 mg/mL of albumin), 25 mL of acetate buffer (50 mM, pH 7.0), 15 mL of acetate buffer (pH 4.5), and 10 mL of extracts were combined, andthe mixture was incubated for 10 min at 37 °C. Then, 25 μL of HA was added, along with 0.3 mg/mL of an acetate buffer with a pH of 4.5, and incubated for 45 minutes at 37 °C. In order to precipitate the undigested HA, 200 μL of 2.5% CTAB in 2% NaOH (pH 12) were added. The mixture was left to sit at room temperature for 10 minutes. The absorbance at λ = 600 nm was used to calculate the turbidity of the reaction mixture. Nine distinct results were obtained from three separate experiments that were each conducted in triplicate. The following equation was used to compute the inhibition percentage:

where TS—absorbance of the enzyme + HA + extract, TC—absorbance of the enzyme + HA, and TH—absorbance of the HA + extract. The data is shown as IC50 values, which represent the extract concentration necessary to inhibit hyaluronidase by 50%. The ANOVA test was run using Statistica 12.0 software to confirm the statistical significance of the data.

Effect on Cyclooxygenase-2 (COX-2) Activity

The COX-2 enzyme (Human recombinant, Cayman No. 60122, pre-diluted 100-fold using 100 mM, pH 8.0 Tris solution) was mixed with reagents from the Cayman COX-2 Assay Kit and prepared as directed by the manufacturer. The tested sample was mixed with 0.12 mL of Tris buffer (100 mM, pH 8.0), 0.01 mL of hemin, and 0.01 mL of the mixture was agitated for five minutes at 25 °C before the addition of 0.02 mL of colorimetric substrate and 0.02 mL of an arachidonic acid solution. The reaction was then started by adding 0.02 mL of COX-2 solution. At 590 nm, the increase of absorbance during the incubation at room temperature was determined. Both a positive sample (the COX-2 inhibitor DuP-697) and a negative (blank) sample (buffer rather than the sample under examination) were run simultaneously. In the computations, the background of the tested samples (0.04 mL sample + 0.19 mL buffer) was also

measured. At least four tests were performed on every sample. When compared to the negative-blank sample, for which the maximal activity was considered to be 100%, the percentage of inhibition of enzyme activity was computed (represented by how many percent the activity was lowered in relation to the negative-blank sample). Additionally, the comparable concentration of acetylsalicylic acid (mg/cm3) in the samples was used to represent enzyme inhibition. For this reason, acetylsalicylic acid solutions were made at 14 concentrations (0.2–10 mg/cm3) and analysed in the same manner as the tested samples.

2. Microbiological Activity

Inoculum Standardization

All microorganism strains were inoculated into Müeller-Hinton broth (pH 7.4) for roughly 16 hours. The suspensions' concentration was adjusted to 0.5 (optical density) using aspectrophotometer.

Assay of Antibacterial Activity Using Agar Well Diffusion Method

The antibacterial activity of both the crude and solvent extracts was evaluated using the Agar well diffusion technique [27]. Sterilized nutritional agar (20 mL) was placed in sterile Petri plates. After each isolate's standardized inoculate had solidified, 100 µL of it was dispersed using sterile spreaders onto nutrient agar plates. The wells were made over the agar plates using a sterile gel puncher with a 6 mm diameter. 100 l of each extract were then added to different wells. As a solvent extract negative control, the extracts were dissolved in 0.9% (v/v) NaCl. Four different concentrations of aqueous and extract were looked at. At 37 °C, the plates were incubated for 24 hours. For each microorganism strain, triplicate of the experiment were saved to assure dependability. After incubation, the diameter of the circular inhibitory zones that formed around each well was measured in mm.

Traditional Uses

Fruits and plants that bear fruit have long been thought to have a variety of health-improving and immunity-boosting effects. Romans used tea made from its leaves to treat a variety of illnesses [28]. Since 8,000 B.C., R. fruticosus has been used as food, and soon after the Ice Age, as a medicinal herb [29]. Hippocrates advised using white wine-soaked blackberry stems and leaves as an astringent treatment on wounds and to ease labor pains [30]. In order to cure mouth ulcers, sore throats, and gum inflammations externally, it is used as a gargle [31, 32]. Leaf decoction is employed as a mouthwash or gargle and to cure thrush [33]. Asthma is treated with the fruit juice [34]. Various respiratory issues can also be treated using the plant's leaves [35]. Blackberry juice is advised for colitis, while tea produced from the berries' roots is used to ease labor pains. Applying a poultice made of leaves to skin lesions. Fruit and juice are advised for anemic patients. As a cicatrizing agent, R. fruticosus leaves or maceration of the tips in sunshine are suggested [36]. The aerial portions have been extracted in methanol and used as an antiseptic, disinfectant, and cough remedy [37, 38]. Cattle skin wounds can be healed by R. fruticosus [39]. The twig tops' decoction is used to cure diarrhea and to calm menstrual cramps. To strengthen the gums and treat thrush, its leaves are eaten. To prevent skin abscesses and fungus infections, leaves are wrapped [40]. Children's throat conditions and diarrhea are treated with R. fruticosus jams that are made without sugar [41]. The roots and leaves have purifying, potently astringent, tonifying, vulnerary, and diuretic

properties. It is a highly effective treatment for hemorrhoids, cystitis, dysentery, and diarrhea [34, 40, 41].

Conclusion

It was discovered that the amount of the examined phytochemicals in blackberry leaf extractsvaries depending on the extract type, which is connected to the solubility of particular constituents.

Based on both free radical scavenging and metal-chelating activity, research has been reviewed the comprehensive antioxidant activity of extracts. Anti-inflammatory and antibacterial activities have also been reviewed. The Loch Tay variety has been chosen as having the highest potential for biological activity, and its use in medicine may be further researched. Based on various tests, it has been shown that all evaluated variations have a wide range of biological activity. It is reasonable to suppose that using the leaves will have similar health benefits to eating fruit while lowering production costs and serving as a source of agri-food waste. As a result, blackberry leaves may be a useful new functional food and a source for the creation of brand-new medicinal formulations with standardized extracts.

R. fruticosus plants are common in colder regions of the planet. It is frequently used in medicine. Cancer, dysentery, diarrhoea, whooping cough, colitis, toothache, anaemia, psoriasis, sore throat, mouth ulcer, mouthwash, haemorrhoids, and mild bleeding can all be treated with various blackberry plants. Numerous pharmacological effects of R. fruticosus include anticancer, antibacterial, antioxidant, antidysentery, antidiabetic, and antidiarrheal properties. The different qualities of "Rubus fruticosus" and the pharmacological effects of theplant's various phytochemical components, including alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, sterols, and carbohydrates, have been addressed in this review article. Ascorbic acid, organic acids, tannins, and volatile oils are also present.

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