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Comparative study of qualitative flavonoid content of *Acacia catechu* bark extracts

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Abstract

Plants include a family of secondary metabolites called flavonoids, which are often included in human diets. Many of these bases have been shown to have positive medicinal potential. The purpose of this investigation was to determine if flavonoids were present in various test extracts from test plant samples. Thirty separate samples of *Acacia catechu* bark were gathered for this study from various seasonal circumstances in the research location. Six extracts were then prepared from each sample. The presence or absence of flavonoids in these extracts was then examined using various techniques. The test sample extracts in ethanol, methanol, aqueous, acetone, and chloroform were found to contain flavonoids, however the benzene extracts were found to be free of the same. Based on the dark-colored reaction mixture, it was claimed that the flavonoids content of both alcoholic extracts was relatively higher. This exploratory study could provide the foundation for future investigations into the therapeutic uses of native plants.

Keywords: *Acacia catechu* bark extract; Flavonoids; Qualitative analysis; Ethanolic extracts; Shinod's test method; Benzene extract

1. Introduction

Secondary metabolites called flavonoids are mostly made up of a benzopyrone ring with phenolic or polyphenolic groups arranged at various locations [1]. Fruits, herbs, stalks, grains, nuts, vegetables, flowers, and seeds are the most frequent places to find them. More than 10,000 flavonoid compounds have been found and identified so far, and the majority of these flavonoids are recognised as effective medicinal substances [2]. These are produced spontaneously via the phenylpropanoid pathway, and their bioactivity is reliant on their bioavailability and mode of absorption [3]. As anticancer, antibacterial, antiviral, antiangiogenic, antimalarial, antioxidant, neuroprotective, antitumor, and anti-proliferative medicines, flavonoids have been widely used. Flavonoid-rich apple peel extracts are an efficient antihypertensive agent and inhibit acetylcholinesterase (ACE) in vitro. Along with preventing cardio-metabolic problems [2-4].

Despite the fact that herbal treatments have been used for a very long time—far longer than modern medicine—little to nothing is known about the safety of utilizing them or the pharmacological basis of their actions [5]. India is one of the biggest hubs and centers for the traditional knowledge of herbal treatments [6]. Many ancient herbal remedies have been examined and refined pharmacologically throughout the years in India, and finally they are included into the official health care system. Numerous secondary plant metabolites with economic value are used in various medications [5-7].

As mentioned in earlier chapters, *Acacia catechu* is a member of the Mimosaceae family and is well-known for a variety of therapeutic qualities. Here, the therapeutic qualities of plant extracts from the bark of *Acacia catechu* in the Guna region have been investigated. The investigation was carried out for preliminary research as well, as no prior data were

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provided [8]. Plant samples were subjected to a qualitative phytochemical screening as part of this investigation utilizing several standardized techniques [5, 7].

Previous studies have also shown the reciprocal relationship of oxidative stress and physical sickness, leading to a multitude of pathological problems [9]. Many individuals choose ayurvedic medications over conventional ones in order to avoid or treat these issues. Herbs have long been utilized as anti-hyperglycemic, anti-obesity, anti-cancer, anti-aging, and anti-oxidative agents because they are rich sources of polyphenols, flavonoids, saponins, and other compounds that act as safer, natural protective agents [8]. Nonetheless, a number of plant species, such as the bark of *Acacia catechu*, are often used as remedies in the Guna area despite the lack of scientific backing for their usage [8-10]. There is a lack of published data, particularly concerning the screening of the flavonoid content of the same. Therefore, it has been investigated if flavonoids are present in various solvent-based extracts of this plant bark made using various techniques.

Widely spread, *Acacia catechu* is a deciduous tree species that belongs to the Fabaceae family. The tree's vertical size ranges from 9 to 12 meters, but its blossoms are coloured light yellow [11]. In addition, the plant specimen has cylindrical spikes that develop into fruits with rectangular pods and a flat morphology. The leaves of the tree are bipinnately complex, with 50 pairs of leaflets that resemble graceful feathers, and small, curving spines. Bark is a thick coating of skin that covers the stems and comes in a variety of colors, from brown to grey [8].

Table 1 Classification of *Acacia catechu*

Kingdom	**Plantae**
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Fabales
Family	Fabaceae
Genus	Acacia Mill.
Species	*Acacia catechu* wild – Black cutch

Table 2 The Different bioactive Flavonoid compounds and their medicinal properties are listed below

Phyto-chemical	**Bioactive compounds**	**Related Medicinal activities**	**References**
Flavonoids	Catechin, Epigallocatechin, Epicatechin gallate, Epicatechin, Epigallocatechin gallate, Eocatechin, Catecutannic acid, Quercetin, Quercitrin, Phloroglucinol, Procatechuic lupenone, Lupeol, Procyanidin AC Quercitrin acid, etc	Antioxidative, Anti-inflammatory, Antipyretic, Anticancer, Anti-ulcer, Skin diseases, Melancholia, Conjunctivitis, Diabetes, Hepato-protective activity, Cough, Pruritus, Leprosy, Body surface infection, Dysentery, Foul Ulcers Wound treatment, Haemorrhage, Anaemia	[3, 7, 12, 14]

The botanical specimen that is being examined belongs to the category of trees, which are mostly found in temperate and semi-temperate areas. *Acacia catechu* is found across much of India, with the exception of areas that are characterized by high humidity, very low temperatures, and desert weather. This is widely distributed geographically among several Madhya Pradesh districts, including Dhar, Rajgarh, Barwani, Guna, Ashoknagar, Harda, Khargone, and Chhatarpur. The Guna location was chosen for our current study project due to the population's high tree density [12].

2. Materials and methods

2.1. Chemicals

Sodium nitroprusside, Phosphoric acid, Ethyl acetate, n-Hexane, Ethyl Ether, Ethanol, benzene, methanol, Sulphuric acid, Sodium bisulphate monohydrate, were supplied by Hi Media Laboratories Ltd., Mumbai, India. Ferrous sulphate, Sodium chloride, sodium sulphate anhydrous, sodium hydrogen sulphate, sodium hydroxide, Sodium nitrate, Ferric chloride, hydrochloric acid, hydrogen peroxide, Iodine, Glacial acetic acid, and all other reagents were purchased from Sisco Research Laboratories (SRL) Pvt. Ltd and from E-Merck (India) Ltd., Mumbai, India.

2.2. Collection and processing of bark samples

The *Acacia catechu* bark samples were randomly gathered from trees in the hamlet of Biloniya, Guna (Madhya Pradesh). A one-kilometer circle was included in the collection area. The selection of bark was always done at a vertical distance of 1.3 meters above ground level in order to preserve uniformity [11]. Samples of bark that were all round or intact were collected, cleaned by hand, and then their weight was determined using a portable digital scale. Three distinct seasons were seen from the collection of five plant samples: winter (especially in mid-January), summer (especially in mid-May), and rainy season (especially in mid-September). The technique used to collect the data covered the period of two years in a row, 2016 and 2017. The materials that had been desiccated in a darkened area were ground at room temperature using a mechanical grinding device in a controlled laboratory setting. They were then filtered through a fine mesh screen with a 0.5 mm pore diameter. After that, 4 °C was the storage temperature for the powdered samples [12, 13].

2.3. Preparation of various bark extracts

The test specimen was split into several aliquots according to procedure. The aqueous extract was extracted from 50 grammes of powdered bark in 1000 milliliters of double-distilled water [11, 12]. Over 3 hours, a magnetic stirrer agitated the extraction under ambient conditions. As before, the mixture was left alone for 24 hours. The filtrate was desiccated and weighed thereafter. The organic solvents (80% ethanol, methanol, benzene, chloroform, and acetone) were prepared by mixing 50 grammes of dried fine powder from the samples with 1000 milliliters at room temperature. After extraction, all samples were dried [13]. Dried extracts were kept at 4 °C in a fridge. To aid analysis, stock extracts were prepared by dissolving desiccated extracts in distilled deionized water (DDW) at 1000 µg/ml throughout the experimental phase.

2.4. Qualitative analysis of flavonoids

The sample extracts were subjected to various qualitative chemical tests using standard protocols as mentioned below.

- **Shinod's test:** This test involves adding a piece of magnesium ribbon and about 400 µl of diluted HCl to a test tube containing one milliliter of sample extract. The presence of flavonoids was detected within a few minutes by the creation of a rich pink or tomato red colour [10, 13].
- **Alkaline reagent test:** This test included adding two milliliters of sample extract to a test tube along with two to three drops of sodium hydroxide. The presence of flavonoids was revealed by the emergence of a rich yellow hue that went colorless when a few drops of diluted HCl were added [13].

3. Results and discussion

The mark "+" indicated the existence of phytochemicals that have been examined. The usage of the '++' symbol denoted a somewhat higher concentration of phytochemicals, while the '+++' sign suggested a much higher concentration. Table 3 displays qualitative testing for flavonoids in various sample extracts using Shinod's test technique. This showed that flavonoids were present in five extracts: methanolic, ethanolic, aqueous, acetone, and chloroform extracts. However, extract made in benzene was shown to be devoid of flavonoids in practically all examined samples. In terms of greater colour intensity than the other extracts, it was observed that the ethanolic, methanolic, and acetone extracts had relatively higher quantities of the same as the aqueous and chloroform extracts.

According to Shinod's test method, plant samples taken in both the 2016 and 2017 years had lower concentrations of flavonoid content in the winter (samples 1–5 & 16–20) and rainy (samples 11–15 & 25–30) seasons than in the summer (samples 6–10 & 21–25). However, it was shown that the methanolic, ethanolic, and acetone extracts from samples 6–10 and 21–25 had larger concentrations of flavonoids than the samples from the winter and rainy season. No, there was a variation in the test parameter's existence between samples taken during the same season in 2016 and 2017. Even yet, the colour intensity in the chloroform extract was found to be greater than in the alkaloid tests, suggesting that the samples may have included more flavonoids than alkaloids. Table 4 shows that the content of flavonoids in aqueous extract was found to be lower than that of chloroform extract for samples taken during the summer months of both the 2016 and 2017 years.

For the same materials, the Shinod's test technique produced results that were significantly different from the qualitative tests for flavonoids conducted using the Alkaline Reagent Test method. In this case, larger concentrations of flavonoids were found in methanolic, ethanolic, and acetone extracts in all analyzed samples across all seasons. But compared to the other season samples, the summer season samples may have shown a somewhat higher quantity of the same. As a result, no seasonal change was seen in the samples for the aforementioned extracts in this assay technique. For samples from the winter and rainy seasons, both aqueous and chloroform extracts were found to contain modest flavonoid concentrations; however, samples from the summer season for the years 2016 and 2017 showed a much greater concentration in terms of colour intensity. It was observed that all benzene samples included zero or very little flavonoids.

Table 3 Qualitative tests for flavonoids using Shinod's test method in different extracts of samples

Tests for flavonoids: Shinod's test						
Sample types	**Types of extracts**					
	Meth	**Etha**	**Aque**	**Ace**	**Chlo**	**Bez**
Sample 1	++	++	+	+++	+	-
Sample 2	++	++	+	++	+	-
Sample 3	++	++	+	++	+	-
Sample 4	++	++	+	+	+	-
Sample 5	+	++	+	+	+	-
Sample 6	++	+++	+	++	++	-
Sample 7	++	++	+	++	++	-
Sample 8	++	++	+	++	+	-
Sample 9	++	++	+	++	+	-
Sample 10	++	++	+	+	++	-
Sample 11	++	++	+	++	+	-
Sample 12	+	++	+	++	+	-
Sample 13	++	++	+	+	+	-
Sample 14	+	++	+	++	+	-
Sample 15	+	++	+	+	+	-
Sample 16	++	++	+	+++	+	-
Sample 17	++	++	+	++	+	-
Sample 18	++	++	+	+	+	-
Sample 19	++	++	+	+	+	-
Sample 20	++	+	+	++	+	-
Sample 21	++	++	+	++	++	-

Sample 22	++	++	+	++	++	-
Sample 23	++	++	+	+++	+	-
Sample 24	++	++	+	+	++	-
Sample 25	++	++	+	++	+	-
Sample 26	+	++	+	++	+	-
Sample 27	++	++	+	+	+	-
Sample 28	+	++	+	++	+	-
Sample 29	+	++	+	+	+	-
Sample 30	+	++	+	+	+	-

Types of extracts – Math (methanolic extract), Etha (Ethanolic extract), Aque (Aqueous extract), Ace (Acetone extract), Chlo (Extract in chloroform), Bez (Extract in benzene). Sample 1-5 were collected in winter (January); 6-10 in summer (May) and 11-15 rainy season (September) in year 2016; Sample 16-20 were collected in winter, 21-25 in summer and 26-30 rainy season in year 2017.

Table 4 Qualitative tests for flavonoids using Alkaline reagent test method in different extracts of samples

Tests for flavonoids: Alkaline reagent test						
Sample types	**Types of extracts**					
	Meth	**Etha**	**Aque**	**Ace**	**Chlo**	**Bez**
Sample 1	++	++	+	+++	+	-
Sample 2	++	++	+	++	+	-
Sample 3	++	+++	+	++	+	-
Sample 4	++	++	+	+	+	-
Sample 5	++	++	+	+	+	-
Sample 6	++	+++	++	+++	++	-
Sample 7	++	++	+	++	++	-
Sample 8	++	+++	+	++	+	-
Sample 9	++	++	++	+++	+	-
Sample 10	++	++	+	+	++	-
Sample 11	++	++	+	++	+	-
Sample 12	++	++	+	++	+	-
Sample 13	++	++	+	++	+	-
Sample 14	++	+++	+	++	+	-
Sample 15	++	++	+	+	+	-
Sample 16	++	++	+	++	+	-
Sample 17	++	+++	+	++	+	-
Sample 18	++	++	+	++	+	-
Sample 19	++	++	+	+	+	-
Sample 20	++	+++	++	+++	++	-
Sample 21	++	++	+	++	++	-

Sample 22	++	+++	+	++	+	-
Sample 23	++	++	+	+++	+	-
Sample 24	++	++	+	+	++	-
Sample 25	++	++	+	++	+	-
Sample 26	++	++	+	++	+	-
Sample 27	++	++	+	++	+	-
Sample 28	++	+++	+	++	+	-
Sample 29	++	++	+	++	+	-
Sample 30	++	++	+	+++	+	-

Types of extracts – Math (methanolic extract), Etha (Ethanolic extract), Aque (Aqueous extract), Ace (Acetone extract), Chlo (Extract in chloroform), Bez (Extract in benzene). Sample 1-5 were collected in winter (January); 6-10 in summer (May) and 11-15 rainy season (September) in year 2016; Sample 16-20 were collected in winter, 21-25 in summer and 26-30 rainy season in year 2017.

For the same materials, the Shinod's test technique produced results that were significantly different from the qualitative tests for flavonoids conducted using the Alkaline Reagent Test method. In this case, larger concentrations of flavonoids were found in methanolic, ethanolic, and acetone extracts in all analyzed samples across all seasons. But compared to the other season samples, the summer season samples may have shown a somewhat higher quantity of the same. As a result, no seasonal change was seen in the samples for the aforementioned extracts in this assay technique. For samples from the winter and rainy seasons, both aqueous and chloroform extracts were found to contain modest flavonoid concentrations; however, samples from the summer season for the years 2016 and 2017 showed a much greater concentration in terms of colour intensity. It was observed that all benzene samples included zero or very little flavonoids. Similar to this study, past research has shown that the bark of *Acacia catechu* trees from different areas contains flavonoids. According to some reports, a strong extract of *Acacia catechu* heartwood contained 66.9% known flavonoid catechin and 23.1% known flavonoid epicatechin, which together made up 90% of the extract's composition. Similar to this, a powdered sample of *Acacia catechu* has a significant quantity of flavonoids [12].

Study after study has shown that flavonoids are a type of polyphenolic compounds that are produced by plants as secondary metabolites and serve a multitude of biological purposes for the plant itself, including growth, development, and fruit ripening [6]. Furthermore, it has been shown that flavonoids shield plants from several biotic and abiotic stressors [9]. Additionally, some research suggested that flavonoids could shield plants against viruses, bacteria, fungi, and herbivores. From a human standpoint, these are strong bioactive substances that are often included in diets [11].

Numerous studies have shown the various flavonoid's anti-inflammatory, antibacterial, and antioxidant properties, which also reduce the risk of disease [6, 8]. Flavonoid antioxidant activity may guard against damage caused by free radicals by the scavenging of reactive oxygen species, activation of antioxidant enzymes, and inhibition of certain damage-causing oxidases. For instance, quercetin, a plant flavonoid that is found in many acacia species, is a well-known antioxidant and has been shown to provide protection against a variety of diseases [12, 14]. Higher concentrations of quercetin have also been detected in red onions' outermost rings and the region closest to the root, excluding *Acacia* species [2, 10]. One study found that organically grown tomatoes had 79% more quercetin than fruit grown non-organically [7]. Flavan-3-ols, also known as flavanols, are a subclass of flavonoids that include pro-anthocyanidins, epicatechin gallate, epigallocatechin gallate, and catechin, among many other structurally diverse compounds [3, 12]. These have previously been reported in several *Acacia* species. Gallate residue in gallocatechol, or gallo-catechin (GC), for instance, and catechin have been shown to function as antioxidant supplements in plants. These results corroborate the current findings [14].

Flavonols, such as kaempferol, quercetin, fisetin, isorhamnetin, and myricetin, are abundant in other plant components including grains, fruits, and young leaves. For example, foods rich in kaempferol and quercetin include red pepper, apples, mangoes, peaches, blueberries, cranberries, and lettuce [4]. Celery, for example, has high levels of apigenin 7-O-glycoside, a flavonoid derivative, whereas numerous citrus fruits, peppers, broccoli, lettuce, cacao, oregano, and other foods have considerable amounts of luteolin and apigenin glycosides of flavonoids [1,4].

Consuming foods rich in flavonoids may help prevent many chronic diseases, as was previously reported. Scientific study has shown that flavonoids have a range of good benefits on human health. For instance, epigallocatechin-3-gallate, a major flavonoid found in green tea, may have a part in the arrest and eventual death of prostate cancer cell

proliferation [1, 5, 12]. Without a doubt, the most significant effect against cancer cells is the capacity of flavonoids to function as antioxidants and scavenge free radicals [11].

These have anti-microbial qualities in addition to the health advantages already described. Studies have indicated that flavonoids can disrupt bacterial membranes, which can lead to the disruption of lipid bilayers and hinder various activities such as the formation of biofilms, the creation of cell envelopes, the inhibition of nucleic acid synthesis, the inhibition of the electron transport chain, and the generation of ATP by microbial agents [9-11]. Quercetin, for example, and the flavonoids epicatechin, epigallocatechin, and gallate seem to trigger an oxidative burst, increasing the production of reactive oxygen species that lead to membrane leakage in microbial cells, which is the outcome of these flavonoids' anti-microbial properties [13, 14]. All extracts in the current analysis demonstrated the presence of flavonoids, with the exception of the benzene extract.

4. Conclusion

Numerous flavonoids have been identified by these studies. This plant specimen possibly proves to have a great deal of medicinal value and a wide range of therapeutic benefits. This research includes plants that contain alkaloids and are utilized as functional foods, herbal medicines, or botanicals, as well as species that produce compounds for test plant medication. This study also shows how difficult it is to evaluate vast datasets, especially in biodiversity and medical development. Finding the causes of variances might be difficult.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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