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Original Article

COMPARATIVE ANALYSIS OF PHYTOCHEMICALS IN *ACACIA CATECHU* BARK EXTRACTS FROM GUNA DISTRICT, MADHYA PRADESH

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ABSTRACT

Objective: Current study aims to discover novel sources of physiologically active natural chemicals with diverse applications. In this study, four types of specific phytochemical in different extracts of *Acacia catechu* bark samples were investigated, which were collected from. Additionally, bibliographic analysis was conducted using dimensions research database.

Methods: For this phytochemical screening, some common and standard test methods were done. Qualitative test for Phenols was done using Lead acetate test method; proteins were quantified by biuret and ninhydrin method, carbohydrates by Molish test, benedict's test and Fehling's test, and starch by using Iodine test method.

Results: Phytochemical screening showed the phenolic compounds (different colour intensity in different samples) and soluble carbohydrates (almost same colour intensity in all test samples) are present and both proteins and insoluble carbohydrate (starch) are completely absent in all test extracts.

Conclusion: The bibliographic analysis indicated the therapeutic values as well as the unavailability of published studies with these special parameters. This preliminary study provide base to find out the possible medicinal significance of *Acacia catechu* of Guna district and also serve as the base for further investigations.

Keywords: Phytochemical screening, Phenols, *Acacia catechu* bark, Bibliographic analysis, Soluble carbohydrates, Herbal therapy

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INTRODUCTION

Plant chemicals are diverse, but the majority fall into four main biochemical categories: polysaccharides, alkaloids, polyphenols, and terpenes [1]. Medicinal herbs are frequently employed in non-industrialized countries, owing to their ease of availability and lower cost than contemporary medications [2]. There are many different types of plants in India that may be utilized medicinally. The ancient Indian literature known as the "Artharva Veda," along with the contributions of several ancient Indian scholars such as "Charak" and "Sushrita," amongst others, were essential to the process of documenting Indian medicinal plants. Because of this, the history of herbal medicine in India is rather ancient [3].

Acacia catechu is broadly cultivated and is a type of deciduous tree that is a member of the Fabaceae family. For many years, Ayurvedic practitioners have made extensive use of this tree in the treatment and prevention of a broad range of ailments and/or problems [4]. The black catechu, kattha, or *Acacia catechu*, also goes by the name Khadira. The wood and bark of the tree are highly prized for its essential medical qualities and are the primary reason for the plant's widespread popularity. It is a well-known plant that is also referred to as black catechu, black cutch, catecu, cutch tree, dark catechu, and gum catechu. For generations, this plant has been used as a wonderful herb with several medical benefits [4-6].

Plants of the genus acacia are widespread across India, particularly in the state of Madhya Pradesh. The traditional Indian medical practices of Ayurveda, Siddha, and Unani all make use of medicinal plants that are grown in Madhya Pradesh. The floral riches of Guna district contribute significantly to the nation's herbal value [7]. An examination of the available research suggests that despite the significant amount of work that has been done on ethnomedicinal plants in India, there are still certain interior regions, such as the Guna district, that need to be studied thoroughly in order to find new traditional remedies [8].

Polyphenols are a prominent and prevalent category of secondary metabolites found in plants. It has been shown to have a variety of pharmacological effects, including anti-arthritic, antiangiogenic, antibacterial, anticancer, antiulcer, mitochondrial adhesion inhibition, and protein kinase inhibition, among others [7, 9]. These chemicals are intriguing because of their presence and unique characteristics. Though presently, these plants and plant products are using for medicinal purpose. But, the scientific research and documentation on the same is meager [10].

This research attempts to consolidate data on phenolic and other chemicals found in the bark of Acacia catechu plants using various extraction techniques. Furthermore, the analysis also included the examination of the presence or lack of starch, soluble carbohydrates, and proteins. The literature data used in this work were gathered from the years 2000 to 2024. The study aimed to conduct a preliminary phytochemical screening of the extract and assess its significance by comparing it with previous studies.

MATERIALS AND METHODS

Collection and processing of bark samples

Samples were collected from five plants over two consecutive years, 2016 and 2017, over three seasons: winter (January), summer (May), and rainy season (Sep), for a season-by-season comparative study. In 2016, samples were grouped as 1, 2, and 3 for the corresponding seasons, whereas in 2017, they were grouped as 4, 5, and 6 for the respective seasons. Bark samples from Acacia catechu trees were gathered following standard protocol at DBH (Diameter at Breast Height). After collecting the samples, they were cleaned in a sterile manner, dried, weighed, and crushed into powder. The powdered components were stored at a temperature of 4 °C for further tests [11].

Bark extract preparation

Six distinct kinds and polarity of solvents were used to create the varied extracts. Standardized processes were used to create, filter, concentrate, and dehydrate extracts of methanol, ethanol, aqueous, acetone, chloroform, and benzene. Dried extracts were refrigerated

at 4 °C for further processing. Dry powders were dissolved in freshly prepared double-distilled water for both *in vitro* and *in vivo* testing prior to the experiment [12].

Phytochemical screening

The qualitative test for the phenolic compound was done by Lead acetate test. For this, 1 ml sample extract was taken in a test tube, then 1 ml ethanol was added, and then diluted with 2 ml, 20% sulfuric acid. At the end, sodium hydroxide was added drop by drop, the conversion of red-to-blue color indicated the presence of different phenolic compounds in the sample [13]. For proteins two test methods, biuret test (using 3% copper sulphate solution and 100 µl of 10% sodium hydroxide) and ninhydrin test (using 0.2% freshly prepared ninhydrin solution) were applied. Soluble carbohydrates were tested using Molish test (few drops of alcoholic α-naphthol solution+50 µl concentrated Sulphuric acid), Benedict's test (using complex mixture of sodium carbonate, sodium citrate, and copper (II) sulfate pentahydrate), Fehling's test (using 2 ml of Fehling's (A and B) solution) and insoluble carbohydrates were tested using Iodine test method (100 µl of iodine solution saturated with potassium iodine) [12, 14, 15].

Bibliographic study

Here, our aim was to analyze the overall number of publications on the medicinal uses of studied phytochemicals of bark extracts of *Acacia catechu* with reference to the plants of Guna district. For this, the dimensions database was used as the primary source of research publications. Data on the number of research articles per year was collected from 2000 to March 2nd, 2024 (at 8 p. m.) using keywords like 'medicinal uses of phenolic compounds in *Acacia catechu* bark extract of Guna district Madhya Pradesh India', 'medicinal uses of protein in *Acacia catechu* bark extract of Guna district Madhya Pradesh India', 'medicinal uses of carbohydrates in *Acacia catechu* bark extract of Guna district Madhya Pradesh India' [16, 17].

Data presentation and analysis

Presence of tested phytochemical was indicated by '**+**" sign. (Moderately high concentration was shown by '**++**sign', While a much higher concentration of phytochemical was shown by '**+++**sign') whereas absence of tested chemical was indicated by '**–**sign' in tables.

In all below tables, the abbreviation and signs indicated are types of extracts–Meth (methanolic extract), Etha (Ethanolic extract), Aque (Aqueous extract), Ace (Acetone extract), Chlo (Extract in chloroform), Bez (Extract in benzene). Samples 1-5 were collected in winter (January); Samples 6-10 were collected in summer (May); and Samples 11-15 were collected in rainy season (September); in year 2016. Samples 16-20 were collected in winter, samples 21-25 were collected in summer and samples 26-30 were collected in rainy season in year 2017.

RESULTS

Results indicated both the qualitative presence and absence of some of the tested parameters in the test samples. In the qualitative tests for phenols, most of the tested samples of all six extracts were seen to exhibit the presence of phenolic compounds in the samples. In all the tested samples, methanolic, ethanolic, aqueous and acetone extracts were reported for the relatively higher concentration of phenols for samples of summer season and moderate concentration for samples of winter and rainy season for all above-mentioned extracts. On the other hand, the chloroform and benzene extracts showed irregular pattern for the presence and absence of phenolic compounds in tested sample for both 2016 and 2017 y (table 1). These results resemble with the reports of earlier scientists on the *Acacia* plants of other zones. The qualitative tests for proteins using Biuret test method and Ninhydrin test method exhibited complete absence of proteins in test samples of all extracts of all seasons of both 2016 and 2017 y (table 2). Along with this, since, carbohydrates are supposed to play a significant role in disease prevention. The various qualitative tests were applied on the all-test sample to find the presence of different types of sugars. In all three assays, i.e., Molish test, Benedict's test and Fehling's test methods all samples were observed to have carbohydrates in the test samples. In all these assays, almost similar colour intensity i.e., the concentration of test parameter, was observed among all samples of all three seasons of both years (table 3). The qualitative tests for starch using Iodine test method showed the complete absence of starch in all the tested samples of all extracts, revealed that the bark samples of tested *Acacia catechu* bark extracts are devoid of starch (table 4).

Table 1: Qualitative tests for phenols using lead acetate test method in different samples of bark extracts of *Acacia catechu*

Test for phenols: lead acetate 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	+	+	+	+	-	-

Table 2: Qualitative tests for proteins using biuret test method and ninhydrin test method in different samples of bark extracts of *Acacia catechu*

Test for carbohydrates: molish test (M), Benedict's test (B) and Fehling's test (F)																		
Sample types	**Types of extracts**																	
	Meth			**Etha**			**Aque**			**Ace**			**Chlo**			**Bez**		
	M	B	F	M	B	F	M	B	F	M	B	F	M	B	F	M	B	F
Sample 1-5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sample 6-10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sample 11-15	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sample 16-20	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sample 21-25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sample 26-30	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 3: Qualitative tests for carbohydrates using molish test, benedict's test and fehling's test in different samples of bark extracts of *Acacia catechu*

Test for proteins: biuret test (B) and ninhydrin test (N)												
Sample types	**Types of extracts**											
	Meth		**Etha**		**Aque**		**Ace**		**Chlo**		**Bez**	
	B	**N**	**B**	**N**	**B**	**N**	**B**	**N**	**B**	**N**	**B**	**N**
Sample 1-5	-	-	-	-	-	-	-	-	-	-	-	-
Sample 6-10	-	-	-	-	-	-	-	-	-	-	-	-
Sample 11-15	-	-	-	-	-	-	-	-	-	-	-	-
Sample 16-20	-	-	-	-	-	-	-	-	-	-	-	-
Sample 21-25	-	-	-	-	-	-	-	-	-	-	-	-
Sample 26-30	-	-	-	-	-	-	-	-	-	-	-	-

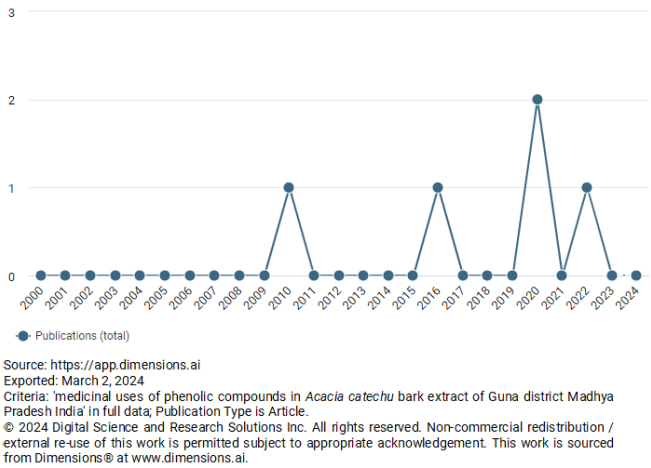

Fig. 1: Database showing number of publications from 2000-2024 on 'medicinal uses of phenolic compounds in *Acacia catechu* bark extract of Guna district Madhya Pradesh India'

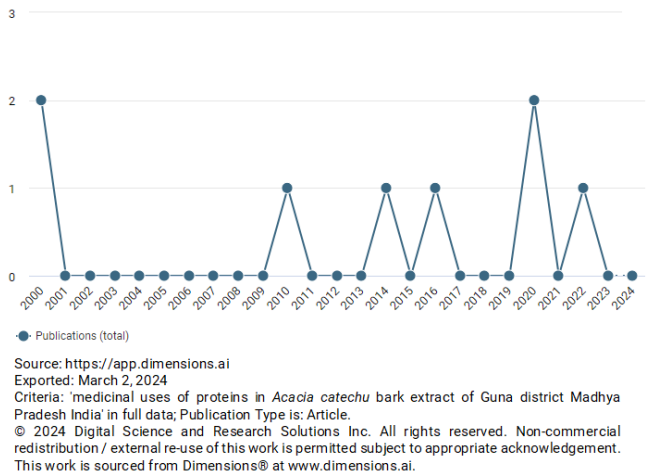

Fig. 2: Database showing number of publications from 2000-2024 on 'medicinal uses of proteins in *Acacia catechu* bark extract of Guna district Madhya Pradesh India'

Table 4: Qualitative tests for starch using iodine test method in different samples of bark extracts of *Acacia catechu*

Test for starch: Iodine test						
Sample types	**Types of extracts**					
	Meth	**Etha**	**Aque**	**Ace**	**Chlo**	**Bez**
Sample 1-5	-	-	-	-	-	-
Sample 6-10	-	-	-	-	-	-
Sample 11-15	-	-	-	-	-	-
Sample 16-20	-	-	-	-	-	-
Sample 21-25	-	-	-	-	-	-
Sample 26-30	-	-	-	-	-	-

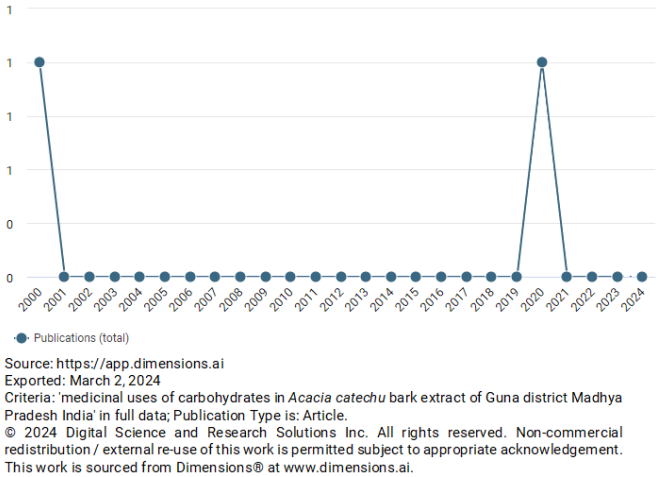

Fig. 3: Database showing number of publications from 2000-2024 on 'medicinal uses of carbohydrates in *Acacia catechu* bark extract of Guna district Madhya Pradesh India'

The bibliographic data showed that there were 5 articles on therapeutic applications of phenolic chemicals, 8 articles on proteins, and 2 articles on carbs in *Acacia catechu* bark extract from Guna district, Madhya Pradesh, India. These few study documents suggested that this plant should be researched for its potential positive activities. The findings showed little literature on the beneficial uses of the bark extract of the test plant in relation to the analyzed parameters.

DISCUSSION

In a number of earlier researches, particularly for catechu bark extract, a correlation between the quality and quantity of secondary metabolites and the therapeutic activities of plant have been noticed [2-4, 6, 13]. Complex combinations of bioactive chemicals are evidently the base of plant-based pharmaceuticals [7].

According to earlier studies plant, based phenolics compounds are secondary metabolites that are very ubiquitous in plants. These are a diverse and wide-ranging aromatic molecule having a benzene ring and one or more hydroxyl groups [18]. Depending on the time of year and the stage of growth and development, different phenolic compounds have different concentrations in plant tissue. Some of these are soluble, while some are insoluble in nature. Some researchers have demonstrated that individuals who consumed the most foods high in polyphenols had an up to 57% reduced chance of acquiring type 2 diabetes than those who consumed the least [5, 19]. Several other studies have also shown a positive connection between polyphenol-rich diets and lower fasting blood sugar levels, improved glucose tolerance, and enhanced insulin sensitivity–all of which are crucial determinants of type 2 diabetes risk reduction. These appear to exert defined physiological functions by either scavenging disease-causing free radicals or by destroying infectious agents from inside the human body. In by both the ways, these actions tend to protect against infectious diseases [8, 14].

Some scientists have studied them for diverse biological actions such as numerous polyphenols, especially phenolic acids, are directly involved in plants stress management. For example, they have been seen to heal damaged plant tissues by lignifying, have antimicrobial properties, and also from heat stress as confirmed by their concentrations in infected areas [16, 19]. Some researchers have found that, phenolic compounds have potent protective efficacy by inhibiting pathogens and decay-causing microorganisms, by preventing triglyceride deposition that may cause heart problems, by reducing the incidence of diabetes, cancer, and stroke, and exerting anti-inflammatory and anti-allergic effects via processes involving reactive oxygen species [20]. Apart from the above-mentioned roles, these are highly beneficial as diet, health supplements, and as medicine too. Many epidemiological studies have shown that diets high in polyphenols reduce the chance of developing chronic human illnesses [21].

Recent studies have proved that the phenolic compounds are abundantly present in *Acacia* species. Many reports revealed the presence of higher amount of the same in fresh vegetables, fruits, green parts of plants, seeds like cereals, tea, coffee, wine, infusions, and so on [22]. It has been studied that outer layers contain more phenolic chemicals than their inner layers of plant body. In order to prevent chain oxidation processes from occurring in biological components, polyphenols, include phenolic groups have the ability to protect against free radicals. For example, it is known that consuming meals and drinks rich in polyphenols may increase the plasma's antioxidant capacity [23]. Consuming antioxidants has been associated with a reduction in the amount of oxidative damage to lymphocyte DNA. Similar results have been found with meals and drinks high in polyphenols, demonstrating the preventive qualities of polyphenols [24].

Recently, some researchers have put light on therapeutic proteins in plants, for example, several proteins extracted from medicinal plants contain anti-microbial, anti-oxidant, anti-HIV, anti-antigen, anti-cancerous, ribosome-inactivating, and neuro-modulatory properties [25]. Though no plant sample can be possible without protein content, as, it is the major component of cell, the absence of proteins in the tested sample possibly revealed the degradation of soluble proteins in the sample before or during the extraction process of the

samples. These finding also similar to that were reported with other scientists. However, some other reports have also demonstrated the presence of low to medium amount of proteins in the bark extracts of the plants, such as, the bark extract of *Terminalia arjuna* has been reported for presence of proteins [12, 19, 26].

Taha *et al.*, (2022), have studied the biological functions performed by proteins in cells. Proteins can be thought of as biomolecular devices with a variety of enzymatic, structural, and functional functions in plants (photosynthesis, respiration, biosynthesis, transport, immunology, reproduction etc.). In order to provide the nutritional and development requirements of growing seedlings, they also serve as storage containers [27]. Typically, these proteins are referred to as storage proteins. Nutritionists are very interested in evaluating the quality and usefulness of proteins from these protein-rich plants since the seeds of *Acacia* plants, which include over 1,350 species, contain a significant quantity of protein (18.25% to 35.5%). Though, in this study, the bark extracts were found to be devoid of proteins [28, 29].

Some researchers have shown that the cell-wall polysaccharide profiles of different fruits and vegetables alter as they mature, store, and are processed, which may indicate that these polysaccharides have vital functions in plants. For example, in a research *Cucurbita maxima* seed have been found to a good source of health-promoting proteins, carbohydrates etc., and were reported to cure benign prostatic hyperplasia. On the other hand, crude protein and carbohydrates were also demonstrated in large amounts in the pumpkin seeds [30].

Since the critical importance of carbohydrates in plants as key sources of energy, carbon skeletons for organic molecules, and storage components is well recognised. They are created during photosynthesis [23, 31]. Additionally, a crucial role as signalling molecules that function similarly to hormones has emerged and is currently being extensively investigated. The presence of carbohydrates in test extracts also supported by earlier researchers in other plants. Since sugars have the ability to scavenge ROS, there is mounting evidence that they serve as antioxidants. Therefore, sugars could be viewed as essential elements of a cohesive cellular redox network [32].

Earlier studies showed the medicinal uses of carbohydrates and also showed that various carbohydrates are currently being researched and tested for their potential to increase resistance. Starch is an insoluble form of polysaccharides [27, 30]. Pure starch is an odor-less, white, amorphous powder that is insoluble in water and other common organic solvents. Due to its role as the energy-storing form of plant materials, it is one of the most extensively dispersed chemical compounds in nature. Due to its inert behaviour, it is insoluble in much of the solvents; this is the possible reason for the negative qualitative results of the same, as also seen in other researches [24, 27, 30, 33, 34].

Additionally, bibliographic studies of these parameters have also put surprising outcome. As given in results very less number of article publication in the area of medicinal uses of phenolic compounds, proteins and carbohydrates in *Acacia catechu* bark extract of Guna district Madhya Pradesh India, respectively [16]. These smaller number of research document indicated that this vegetation needed to be studied for their possible beneficial roles, if any. Though the obtained data revealed some available literature of the beneficial applications of the extracts of the bark extract of the test plant but with reference to the studied parameters data is meager.

CONCLUSION

There are some data that show its wound healing as well as antibacterial/antimicrobial actions of the studied plant. In addition to this the Guna district of central India has dense forest of the same plant. Though, presently, these plants and plant products are using for medicinal purpose. Therefore, for our research work, this highly potent and abundant plant that is Acacia catechu was chosen for investigation. As a preliminary examination, it was essential to research the phytochemistry of this plant, that might helpful to reveal out its possible therapeutic actions. Up to best of our knowledge and available data, this is the first study that revealed the season-wise comparative analysis of the protective action of bark extract. In the same way, this study also a worthful step towards sustainable development and possibly shifts the therapy towards already available herbal-based medicine than synthetic chemicals.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

Declared none

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