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Original Article

STEROIDS AND TRITERPENOIDS IN *ACACIA CATECHU* BARK EXTRACTS: A COMPARATIVE STUDY AND BIBLIOGRAPHIC ANALYSIS

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ABSTRACT

Objective: Plant-derived steroids and triterpenes have been recognized for a number of health benefits. As indicated in previous research, some of the phytosterols are essential dietary substances. This study was aimed at investigating the presence or absence of these phytochemicals in various test bark extracts from *Acacia catechu* plant samples.

Methods: For this study, thirty samples of test plants were gathered in various seasons from the research location. Six different solvents were then prepared for each sample. Then the qualitative tests of the studied parameters were done using various standardized methods. Additionally, bibliographic analysis was done to validate and co-relate the present study with earlier findings.

Results: Results showed the presence of test parameters in all solvent systems except aqueous extracts, though the colour intensity (quantity) was different in all other extracts. Based on the dark-colored reaction mixture, it can be claimed that the steroid and triterpene content was relatively higher in methanolic and ethanolic extracts. The comparative bibliographic study was also validating the beneficial applications of the same.

Conclusion: Possibly, this preliminary study helped to find out the medicinal significance of native flora of Guna district and also serve as the base for further investigations.

Keywords: *Acacia catechu* bark extract, Steroid and triterpene, Qualitative study, Ethanolic extracts, Bibliographic analysis, Phytosterols, Herbal remedies

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INTRODUCTION

Plant-derived herbal remedies are extensively used throughout many locations worldwide [1]. There is enough evidence to support the health advantages of the plant's roots, stems, leaves, bark, and fruits. Phytochemicals found in medicinal plants have pharmacological effects that may mimic the actions of pharmaceuticals, making them suitable for application in modern medicine [2, 3].

Polyphenols, phytoestrogens, anthocyanidins, flavonoids, is flavonoids, terpenoids, triterpenoids, carotenoids, limonoids, tannins, phytosterols, glucosinolates, and fibers are a few of the phytochemicals that have been identified as possibly having health advantages [3, 4]. Phytosterols are common in the plant world as plant steroids. Certain phytosterols have hypocholesterolemic effects, which is significant [5]. Withanolides are a varied group of steroidal lactones that have a variety of biological roles [6]. Triterpenoids are often produced in certain plant tissues or at particular developmental stages, and may also be induced in response to various abiotic and biotic stress factors. Multiple studies have shown that certain triterpenoids are crucial for plant development and defense systems [7]. Plants and fungi both produce triterpenes, such squalene, which acts as the precursor to all steroids. Triterpenoids possess a diverse range of potentially advantageous biological characteristics, including anti-inflammatory, anticarcinogenic, antidiabetic, hepatoprotective, antibacterial, antimycotic, analgesic, immunomodulatory, and cardiotonic actions [3, 7]. Certain triterpenoids possess hemolytic and cytostatic characteristics that might lead to adverse effects, limiting their therapeutic potential. Semisynthetic derivatives of triterpenoids are being used to remedy the issue [8, 9].

For long, *Acacia catechu* has been valued for its therapeutic benefits. It is a plant of historical significance, much used in traditional medicine across the globe [10]. This plant's bark has high antioxidant, astringent, anti-inflammatory, antibacterial, antifungal, wound-healing, and hepatoprotective properties. It is used as a mouthwash for oral hygiene, gum health, throat irritation, gingivitis, and dental infections. Various bioactive compounds such alkaloids, tannins, flavonoids, steroids, and triterpenoids have shown therapeutic properties in many research investigations [3, 8, 9]. To support the significance of present work, the bibliographic analysis has also been done. This is an analysis, interpretation, and discussion of the findings, conclusions, and theoretical stances from scientific papers comprise the research approach. It is a methodical approach to computer-assisted scientific reviews that may pinpoint key findings or writers along with their connections.

This study aimed to evaluate the phytochemical profile with special reference to qualitative analysis of steroids and triterpenoids in different extracts of *Acacia catechu* bark samples collected from Guna region of Madhya Pradesh, India. This region is rich in this flora but to the best of our knowledge and based on accessible bibliographic data, this research is the first to provide a seasonal comparison analysis. This research demonstrated the influence of both season and kinds of solvent system on the parameter under investigation to make optimum use of the extract for therapeutic purposes. Furthermore, this study focused on analyzing the bibliography of phytochemicals of bark extracts from the same plant, with a specific emphasis on research related to steroids and triterpenoids. This analysis provides valuable insights into the scholarly landscape, publication trends, authorship patterns, subject coverage, and citation networks, identify research gaps, and make informed decisions for future research direction [11, 12].

MATERIALS AND METHODS

Collection and processing of bark samples

For the purpose of season-by-season comparison research, samples from five plants were collected for two consecutive years, 2016 and 2017, in each of the three seasons-winter (January), summer (May), and rainy season (September). Samples collected in year 2016 were

considered as groups 1, 2, 3 for the respective above-mentioned seasons) and 2017 were considered as groups 4, 5, 6 for the respective above-mentioned seasons). Following the usual procedure, the bark samples of plants were collected from randomly chosen *Acacia catechu* trees at DBH (Diameter at Breast Height). Following sample collection, they were aseptically cleaned, dried, weighed, and pulverized. After that, the powdered materials were kept for the next experiments at a temperature of 4 °C [13].

Bark extract preparation

Six different types and polarities of solvents were used to prepare the various extracts. Here, standardized procedures were used to produce, filter, concentrate, and dry extracts of methanol, ethanol, aqueous, acetone, chloroform, and benzene. In order to be processed further, dried extracts were kept in a refrigerator at 4 °C. For both *in vitro* and *in vivo* tests, dry powders were dissolved in brand-new double-distilled water at the time of the experiment [10].

Test for steroids and triterpenoids

To get authentic and comparative results for the qualitative analysis of steroids and triterpenes, the following three standardized methods were applied.

(a) Horizon test: To do this, one milliliter of sample extract was extracted, and two milliliters of trichloroacetic acid were added. The formation of a red precipitate in the sample served to confirm the presence of terpenoids [11].

(b) Liebermann burchard's test: To do this, put two milliliters of sample extract in a test tube and add one milliliter of ethanol. This included adding 0.5 ml of chloroform and heating it in a water bath for 10 min. Next, 2 drops of sulfuric acid and 1 ml of acetic acid solution were poured along the test tube's side. The appearance of a violet-blue ring complex at the intersection of the two liquids served as evidence that triterpenoids and steroids were present in the sample [12].

(c) Salkowski's test: Two milliliters of the sample extract and two milliliters of chloroform were combined in a test tube. After that, 1 milliliter of concentrated sulfuric acid was mixed into the mixture. Triterpenes were present when they appeared as greenish-yellow or golden-yellow fluorescence [11, 13].

Bibliographic study

Here, our aim was to analyze the overall number of publications on the phytochemicals of bark extracts of *Acacia catechu* and its comparison with the number of publications on the steroids and triterpenoids of the same. For this, the dimensions database was used as the primary source of research publications. Number of publications per year and co-authorship analysis from 2000 to March 22, 2024 (at 10 a. m.) were performed using keywords like 'phytochemicals of bark extracts of *Acacia catechu*' and 'Steroids and triterpenoids of bark extracts of *Acacia catechu*'. Citation analysis and network visualizations were done with the VOS viewer application. Researchers are classified according to how many articles they have co-authored [14-16].

Data representation and statistical analysis

For analysis of qualitative data of tested phytochemicals, the mark "2" indicated the existence of phytochemicals that have been examined. The usage of the '3' symbol denoted a somewhat higher concentration of phytochemicals, while the '1' sign suggested an absence of the same. The expressed data as mean±standard deviation of all five samples of each group. In table 1, comparative data of each group of different extracts have been given, while table 2 illustrated season-wise comparison of the same. The numeric values were assigned only to indicate the comparative content of test samples. Bibliographic data is given in as graphs and Co-authorship network figures.

RESULTS

The solvent system-wise comparative qualitative tests for steroids and triterpenoids using the Horizon test method revealed the presence of steroids and triterpenoids in the tested samples of all extracts except the aqueous extracts for all samples in all three methods. Both methanolic and ethanolic extracts were reported to exhibit a greater amount of the same in all assay systems (table 1). Other three solvent systems were also seen to express the presence of the same, but no significant concentration variations were seen among them. Season-wise comparative studies revealed that the samples of the summer season showed the greatest, while the samples of the winter were reported to have the least amount of the same (table 2).

Table 1: Comparative qualitative tests for steroids and triterpenoids using Horizon test (H), Liebermann Burchard's test (B) and Salkowski's test (S) method in different solvent system of bark extracts of *Acacia catechu*

Sample	Types of extracts																	
	Meth			Etha			Aque			Ace			Chlo			Bez		
	H	B	S	H	B	S	H	B	S	H	B	S	H	B	S	H	B	S
G1	2±0	2.2±0.45	2±0	2±0	2.2±0.45	2±0	1±0	1±0	1±0	2±0	2±0	2±0	2±0	2±0	2±0	2±0	2±0	2±0
G2	2.2±0.45	2.6±0.55	2±0	2±0	2.6±0.55	2±0	1±0	1±0	1±0	2±0	2±0	2±0	2.4±0.55	2.6±0.55	2±0	2.2±0.45	2.6±0.55	2±0
G3	2±0	2±0	2±0	2±0	2±0	2±0	1±0	1±0	1±0	2±0	2±0	2±0	2±0	2±0	2±0	2±0	2±0	2±0
G4	2±0	2.2	2±0	2±0	2±0	2±0	1±0	1±0	1±0	2±0	2±0	2±0	2±0	2.2±0.45	2±0	2±0	2.2±0.45	2±0
G5	2.1±0.45	2.6±0.55	2±0	2±0	2±0	2±0	1±0	1±0	1±0	2±0	2±0	2±0	2.2±0.45	2±0	2±0	2.1±0	2±0	2±0
G6	2±0	2±0	2±0	2±0	2±0	2±0	1±0	1±0	1±0	2±0	2±0	2±0	2±0	2±0	2±0	2±0	2±0	2±0

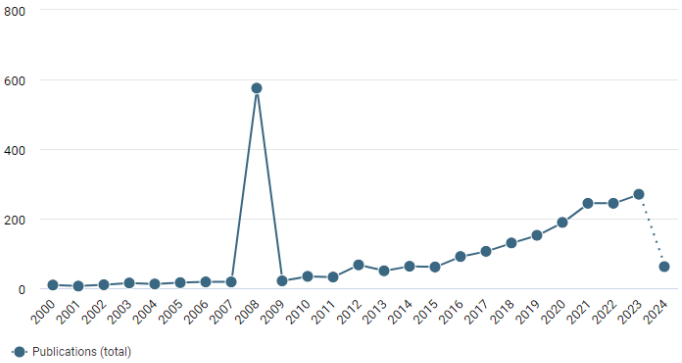
Types of extracts–Meth (Extract in methanol), Etha (Extract in Ethanol), Aque (Extract in Aqueous), Ace (Extract in Acetone), Chlo (Extract in chloroform), Bez (Extract in benzene). G1-G6 designated for Group 1 to Group 6 plants.

Table 2: Comparative qualitative tests for steroids and triterpenoids using Horizon test (H), Liebermann Burchard's test (B) and Salkowski's test (S) method of bark extracts of *Acacia catechu* in different seasons

Sample types	Types of extracts																	
	Meth			Etha			Aque			Ace			Chlo			Bez		
	H	B	S	H	B	S	H	B	S	H	B	S	H	B	S	H	B	S
Winter	2	2.2	2	2	2.1	2	1	1	1	2	2	2	2	2.1	2	2	2.1	2
Summer	2.1	2.5	2	2	2.45	2	1	1	1	2	2	2	2.3	2.4	2	2.1	2.4	2
Manson	2.05	2.2	2	2	2.2	2	1	1	1	2	2	2	2.1	2.15	2	2.05	2.15	2
Net Mean	2.1	2.3	2	2	2.3	2	1	1	1	2	2	2	2.1	2.2	2	2.1	2.2	2

Values are in mean of group 1 and 4 for winter; group 2and5 for summer and group 3and6 for manson seasons. Types of extracts–Meth (Extract in methanol), Etha (Extract in Ethanol), Aque (Extract in Aqueous), Ace (Extract in Acetone), Chlo (Extract in chloroform), Bez (Extract in benzene). G1-G6 designated for Group1 to Group 6 plants and, 'Net mean' indicated total mean values of all seasons (i.e. samples of group 1to group 6) to compare different extracts overall.

Moreover, the bibliographic results indicated a total of 2,590 publications on 'phytochemicals of bark extracts of *Acacia catechu*'. According to this database, from 2002 onwards, the number of publications on the same were increased rapidly (fig. 1). The bibliographic analysis of steroids and triterpenoids in bark extracts of *Acacia catechu* indicated a very limited number of publications until 2005, while after that, these were seen to increase. Though, as compared to total phytochemicals, the publications on our test parameters were seen to be very limited (fig. 2). Also, with reference to the Guna region of Madhya Pradesh, India, the data is meager. With key words "steroids and triterpenoids of bark extracts of *Acacia catechu* of Guna Madhya Pradesh" total 35 publications were reported. Detailed study revealed that from 1975 to 2002 there was zero number of publications on the same; after that also till 2018 the total published reports were 12 (fig. 3). So, these findings indicated that there is a need to study these parameters as preliminary research. Though existing reports indicated therapeutic potential of the studied phytochemicals hence, this investigation could provide a base for further research.



Source: https://app.dimensions.ai
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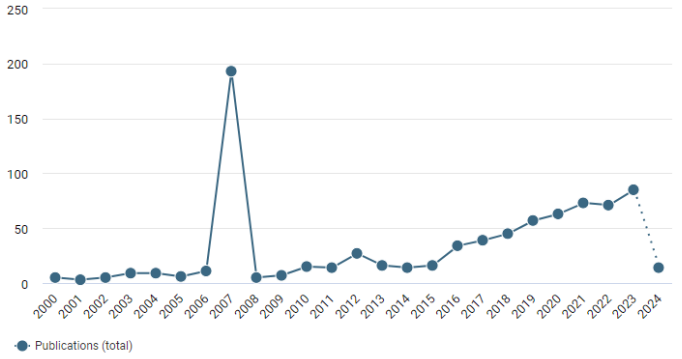
Criteria: 'phytochemicals of bark extracts of Acacia catechu' in full data. © 2024 Digital Science and Research Solutions Inc. All rights reserved. Non-commercial redistribution / external re-use of this work is permitted subject to appropriate acknowledgement. This work is sourced from Dimensions® at www.dimensions.ai.

Fig. 1: Database showing the number of publications from 2000-2024 on 'phytochemicals of bark extracts of *Acacia catechu*'



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Criteria: 'steroids and triterpenoids of bark extracts of *Acacia catechu*' in full data.
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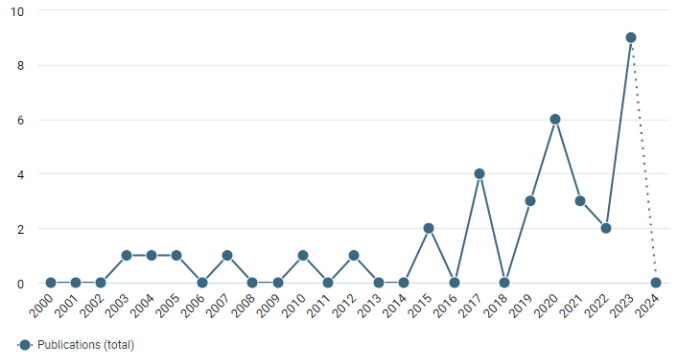
Fig. 2: Database showing number of publications from 2000-2024 on 'steroids and triterpenoids of bark extracts of *Acacia catechu*'



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Criteria: 'steroids and triterpenoids of bark extracts of *Acacia catechu* of Guna madhya pradesh' in full data. © 2024 Digital Science and Research Solutions Inc. All rights reserved. Non-commercial redistribution / external re-use of this work is permitted subject to appropriate acknowledgement. This work is sourced from Dimensions® at www.dimensions.ai.

Fig. 3: Database showing number of publications from 2000-2024 on 'steroids and triterpenoids of bark extracts of *Acacia catechu* of Guna madhya pradesh'

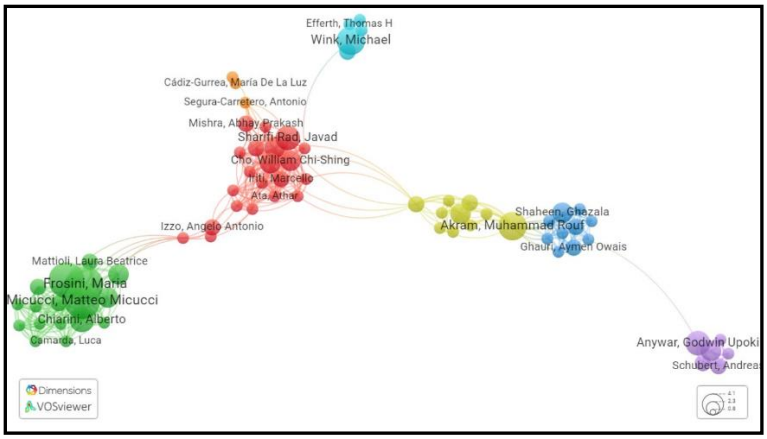

Fig. 4: Co-authorship analysis of database for on 'phytochemicals of bark extracts of *Acacia catechu*'

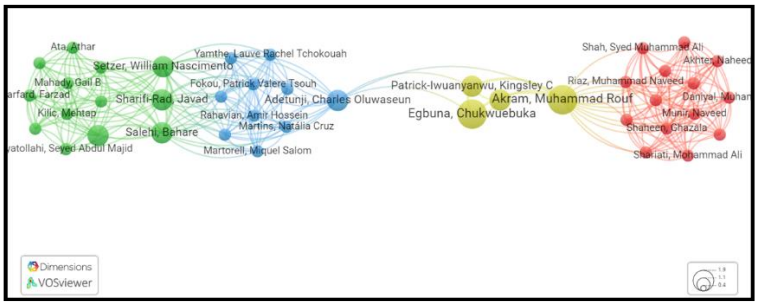

Fig. 5: Co-authorship analysis of database for on 'steroids and triterpenoids of bark extracts of *Acacia catechu*'

In addition to this, using Vos viewer, the relatedness of researchers was also determined based on their number of co-authored publications for both of the above-mentioned keywords. For the key word 'phytochemicals of bark extracts of *Acacia catechu*' a total of 69 researchers were found to exhibit 634 co-authorships and 369 co-authorship links. These have been divided into seven clusters (fig. 3). On the other hand, for the key word 'steroids and triterpenoids of bark extracts of *Acacia catechu*', the number of researchers was recorded to be 41, which showed a total of 273 co-authorships and 267 co-authorship links and can be classified into four clusters (fig. 4). These data again indicated a research gap, with special reference to the test area and tested parameters.

DISCUSSION

Numerous investigations completed since the previous two years have demonstrated thousands of plant's pharmacological benefits [2, 4, 17]. Numerous studies on the therapeutic properties of leaves, seeds, roots, flowers, and woods have also been reported, with particular reference to the *Acacia* species. To the best of our knowledge, there is very little scientific data on the major species in Guna district, *Acacia catechu* plant [13, 18, 19].

As supporting evidence, some prior research on the protective properties of the same plant's seeds, leaves, roots, bark, and heart wood is considered [20, 21]. Furthermore, as additional information, the medicinal properties of certain herbs have been examined in various solvent systems for the treatment of a variety of other illnesses, including diabetes, hepatic illness, cardiovascular issues, viral infections, ulcers, oral cavities, etc. It is anticipated that these reviews will aid in elucidating the potential mechanisms of action of the plant's bioactive components [10, 22, 23].

These findings are consistent with those of previous studies conducted on other *Acacia* species. for instance, examined the phytochemical present in the water extract of the bark of *Acacia seyal*, which had a combination of three steroids-campesterol, stigmasterol, and clionasterol-as well as one triterpene, lupeol [24]. Three triterpene glycosides have been isolated and recognized from *Acacia myrtifolia* leaves. The capacity of many triterpene glycosides produced from *Acacia victoriae* to cause apoptosis and slow the development of tumour cells has recently been investigated. It has been discovered that the pods of *Acacia sinuate* contain the triterpene ester genin. Many species belonging to the Phyllodineae family, such as *Acacia melanoxylon*, *Acacia auriculiformis*, *Acacia meansii* and *Acacia obtusifolia*, have been shown to contain the phytosterols stigmast-7-enol and spinasterol [6, 16]. Conversely, the investigation using *Acacia nilotica* bark extract showed that terpenoids were present but that steroids were not [25]. Chemically speaking, the term "steroid" comes from the word of sterol, a hormone that occurs naturally. Most plant species contain steroids, such as flaxseeds, *Nigella sativa*, *Ocimum gratissimum* Linn leaf, *Syzygium guineans* root, and *Vernonia amygdalina* root and stem bark. Similar to this, terpenoids-tiny chemicals produced by plants-are perhaps the most prevalent class of natural goods [26, 27].

Terpenoids have significant pharmacological effects, such as antiviral, antibacterial, antimalarial, anti-inflammatory, inhibition of cholesterol synthesis, and anti-cancer qualities [6]. The bulk of the plant species analyzed contained terpenoids, such as citrus fruit juice, the leaves and roots of *Hagenia abyssinica*, the leaves and roots of *Leucas aspera* L., flax seeds, the leaves of *Ruta chalepensis*, and the root of *Syzygium guineans*. Nevertheless, the results varied among certain plants based on the type of solvents used [23].

For bibliographic analysis the 'demotions' was chosen because a vast number of papers are covered by the information system Dimensions, which also makes it simple to obtain and analyze datasets. With this, real-time bibliometric analysis may be performed, including co-authorship and citation analysis source title citation analysis [16]. For co-authorship and citation analysis VosViewer was used, because it is a tool, we may use to analyze Dimensions data. A canvas for making a network map using bibliographic data is offered by VosViewer [14]. So, these have been used for the data source and to represent the obtained results [28]. The bibliographic, co-authorship and citation analysis revealed that the overall, there were thousands of studies have been published

that confirmed the various health benefits of *Acacia catechu* bark extracts, with special reference to the steroid and triterpenes also some data available that clarify its applications [29-31]. Hence, phytochemistry and pharmacology of the test phytochemicals needed attention to uncover their beneficial health effects or possible medicinal behaviour, if any.

CONCLUSION

Though many plant-derived phytochemicals have been known for their medicinal applications, The application of steroids and triterpenes is meager. In our test plant and research area, publications are also negligible. Hence, this study provides both qualitative data on these parameters and their available research data. In the present study, except for aqueous extracts, all other extracts showed more or less steroid and triterpene content; further study is needed for their possible health impacts. If these extracts were recognised as potent medicinal molecules, then the abundantly available flora of the Guna district of Madhya Pradesh would be useful as herbal medicines.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

Declared none

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