

A COMPARATIVE STUDY OF POLYPHENOLIC CONTENT IN *ACACIA CATECHU* BARK EXTRACTS AND BIBLIOGRAPHIC ANALYSIS WITH REFERENCE TO GUNA (MADHYA PRADESH), INDIA

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ABSTRACT

Objective: Plant-based polyphenolic compounds are important phytonutrients found in many meals such as fruits, vegetables, tea, coffee, and red wine. The literature study has gathered enough evidence confirming the existence of the same substance in the bark of *Acacia catechu* plants. The literature review verified that the dense forest with comparable plant life in Guna district of Central India has not been systematically studied. This research aims to analyze the total polyphenolic content of *A. catechu* bark from this location.

Methods: Thirty samples of test plants were collected from the research area in different seasons for this investigation. Each sample was made using six distinct solvents. Subsequently, quantitative testing was conducted using a standardized laboratory approach. Bibliographic analysis was conducted to confirm and establish a connection between the current study and previous research.

Results: The results indicated that the test parameter was present in large quantities in all polar extraction systems, but only insignificant amounts were seen in chloroform and benzene. The comparative bibliographic investigation was confirmed the advantageous uses of the same.

Conclusion: This exploratory investigation potentially identified novel, cost-effective, and easily accessible sources of polyphenolic chemicals from the local flora of Guna region and serve as the foundation for future researches on the same.

Keywords: *Acacia catechu*, bark extract, polyphenols, aqueous extract, bibliographic analysis, herbal remedies, seasonal impact on polyphenolic content.

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INTRODUCTION

Nowadays, consuming enough portions of fruits and vegetables, green tea, pure chocolate, millet, and red wine regularly in meals is widely promoted to the public since they are rich in polyphenols and beneficial for maintaining good health [1]. Polyphenols are present in several plant-based foods and drinks, such as apples, grapes, tea, coffee, berries, onions, citrus fruits, fresh leafy veggies, broccoli, plums, millets, chocolate, and more [2]. These are a significant group of phytonutrients known as plant secondary metabolites and considered crucial functional foods or dietary bioactive substances. The major categories of polyphenols are flavonoids, lignans, stilbenes, and phenolic acids, and they have continuously been investigated for their dietary and medicinal applications [2-4].

A class of polyphenolic molecules called flavonols, such as quercetin, is found in onions, apples, parsley, sage, and tea. Anthocyanins are abundant in berries [5]. There is significant epidemiological evidence that these polyphenolic compounds help to prevent many lifestyle-related disorders, including overweight, cardiovascular disease, and Type 2 diabetes; they are also crucial for numerous physiological responses [2]. Polyphenols in the diet aid in enhancing lipid profiles, immunity, blood pressure, insulin resistance, antioxidative system, and systemic inflammation [2,4,5].

Quercetin, catechin, anthocyanin, etc., are physiologically active polyphenols, leading to several human intervention studies examining their effects [2,6]. Scientists are always seeking more readily accessible sources of these types of supportive dietary supplements to meet the needs of a large population. This investigation was likewise carried out in the same manner. The previous research has shown that the bark of

Acacia catechu contains several bioactive molecules, including catechin, kaempferol, quercetin, quercitrin, fisetin, tannins, rutin, epicatechin, catecholamines, ascorbic acid, Certain B vitamins, carotenoids, and various other antioxidants and dietary compounds [7-9]. Some researchers have found an association between the quality and quantity of secondary metabolites in catechu bark extract and its therapeutic actions. For example, flavonoids, including quercetin-3-O-rutinoside and quercitrin, are found in significant concentrations in catechu bark [10].

The bark of this plant contains bioactive molecules that Ayurveda references as having medicinal properties for treating skin diseases, physiological dysfunctions, coughs, diarrhea, cancer, chest discomfort, ulcers, vitiligo, wound healing, etc [7-10]. The *A. catechu* bark has been shown to possess antifungal, antiviral, spasmolytic, hypoglycemic, and anti-inflammatory effects. In a number of studies, *A. catechu* is a rich source of strong antioxidants such as catechins and epicatechins. The same has been reported to exhibit effective anti-oxidative behavior against oxidative stress caused by cancer cells and was proved to be strong anti-cancer drugs. In another study, specific polyphenolic compounds were reported to have excellent antioxidant and anti-cancer properties [6,7,9,11].

The literature research has provided sufficient data supporting the positive activities of *A. catechu* plants [6-11]. Furthermore, the literature assessment confirmed that the thick forest with similar flora in Guna district of central India has not been scientifically researched yet [9]. Thus, the study focused on the abundant and untapped flora found in the Guna region, which belongs to central India. This first analysis was conducted to study the overall polyphenolic content of bark samples. For this, bark samples were comparatively analyzed for effects of

season, various extraction systems time intervals, etc. The obtained quantitative data were gathered and examined at various levels. Furthermore, existing literature and bibliographic analysis have been compiled to enhance the understanding and significance of the current study. This study may provide useful ethnomedicinal information for future studies.

METHODS

Chemicals

The standard flavonoid compound gallic acid of 99.99% purity was purchased from Hi Media Laboratories Ltd. Other chemicals such as phosphoric acid, Dimethyl sulfoxide, solvents such as toluene, ethyl acetate, n-Hexane, ethyl Ether, ethanol, benzene, methanol, and distilled water of research grade were purchased from Hi Media Laboratories Ltd., Mumbai, India. Sodium chloride, sodium sulfate anhydrous, sodium hydrogen sulfate, and all other reagents were purchased from Sisco Research Laboratories Pvt. Ltd and from E-Merck (India) Ltd., Mumbai, India.

Bark samples collection and processing

For this study, total of 30 different plant samples of specimen were deposited in the herbarium of School of studies in Botany, Jiwaji University Gwalior, Madhya Pradesh, (voucher number AC-101A-1010/SOB2016 and AC-102A-1020/SOB 2017) were randomly collected in two consecutive years (i.e., 2016 and 2017) from Biloniya hamlet, Guna (Madhya Pradesh), (the geographical coordinates are L24.650000, A77.320000) in an area of one km. To get seasonal impact on total polyphenolic content, five plants were sampled in winter (mid-January), summer (mid-May), and rainy season (mid-September) each year. 2500–3000 g of bark was randomly taken from *A. catechu* trees at DBH (Diameter at Breast Height) 1.3 m above the ground. Manually cleaning samples with a cotton cloth followed collection. The bark samples were carried to the lab under aseptic conditions and dried in the shade for 4 weeks. These bark samples were then ground at room temperature using a mechanical grinder and strained through 0.5 mm mesh. Powdered materials were kept at 4°C for subsequent experiments [9].

Preparation of bark extracts

At room temperature, 50 g bark powder was extracted with 1000 mL of double distilled water (1/20 w/v) with continuous magnetic stirring for 3 h and left for 24 h to make aqueous extract. The filtrate was dried and weighed. For organic solvent extracts (80% ethanol, methanol, benzene, chloroform, acetone), 50 g of dried fine powder of samples was thoroughly mixed with 1000 mL (1/20 w/v) of solvent at room temperature. After 12 h on a 150-rpm shaker, they were left stationary for 24 h. Solutions were muslin-filtered and re-filtered using Whatman No. 1. Pure extract was obtained by full solvent evaporation under decreased pressure from these filtrates. The dried extracts were refrigerated at 4°C for processing. For *in vitro* and *in vivo* testing, dry powders were dissolved in fresh double distilled water [9,10].

Total polyphenols quantification

For this assay, Jyoti *et al.*, (2003) method was followed. A 0.125 mL sample of a test extract at a concentration of 100 mg/mL was combined with 0.5 mL of distilled water. Next, 0.125 mL of Folin-Ciocalteu reagent was introduced. The volume was finalized at 3.0 mL by adding distilled water and then incubated at room temperature for 90 min. The absorbance was measured at a wavelength of 765 nm by comparing it to a pre-generated blank sample. This comparison was made in relation to a set of standard samples with known quantities of gallic acid. The findings were quantified in milligrams of gallic acid equivalent per 100 g of dry weight of the extract [12,13].

Bibliographic study

Here, our aim was to analyze the overall number of publications of the past 50 years on overall polyphenolic content of *A. catechu* bark extract and also in Guna region. For this, the dimensions database was used as the primary source of research publications. Data on the number of

research articles per year were collected from 1975 to March 5th, 2024 (at 8 p.m.) using keywords polyphenolic content of *A. catechu* bark extract' and "polyphenolic content of *A. catechu* bark extract of Guna district of India" [14,15].

Statistical analysis

The obtained results were analyzed at three bases and divided into three sections. Sec-1: In this section, comparative study of polarity and nature of solvent on the extraction of total polyphenolic content was calculated. Sec-2: In this section, data were analyzed for comparative seasonal-wise analysis, where in the same samples, the impact of three seasons, (i.e., winter, summer, and monsoon) on total polyphenolic content was calculated. Sec-3: In this section, samples of six different groups of individual solvent system (i.e., samples collected in the same seasons of different years) were compared for differences in their total polyphenolic content, if any. The values are expressed in mean \pm SE. A one-way analysis of variance was conducted, followed by an unpaired Student's t-test. A significance level of 5% or less was taken as significant [12,13].

Data presentation

In all the below figures, the abbreviation and signs indicated are types of extracts-Meth (methanolic extract), Eth (Ethanol extract), Aqu (Aqueous extract), Ace (Acetone extract), and Chlo (Extract in chloroform), Benz (Extract in benzene). Samples 1–5 were collected in winter (January 2016) represent as group-1 (G1); Samples 6–10 were collected in summer (May 2016) represent as group-2 (G2); and Samples 11–15 were collected in rainy season (September 2016) represent as group-3 (G3); Samples 16–20 represent as group-4 (G4); were collected in winter (January 2017); samples 21–25 were collected in summer (May 2017) represent as group-5 (G5); and samples 26–30 were collected in the rainy season (September 2017) represent as group-6 (G6).

RESULTS

Results showed a high to low amount of total polyphenolic content in different extracts. These results were calculated on different bases, as mentioned above. Analysis of sec-1 showed that the extraction of bark samples in methanol and ethanol solvents demonstrated an equal amount of polyphenolic content, while extraction in aqueous medium showed a significantly greater amount of the same ($p < 0.05$) than all other extraction systems. Both chloroform and benzene showed negligible amount of total polyphenols, indicating that possibly these cannot be referred to as good extraction mediums. However, acetone showed a remarkable lesser ($p < 0.05$) polyphenolic content than methanol, ethanol, and aqueous medium (Fig. 1).

The results of the Sec-2 analysis are given in Fig. 2. In this graph, the comparison of the three studied seasons for all extracts has been taken. For this calculation, plant samples collected in two consecutive years (i.e., five samples from 2016 and five samples from 2017) were added to collect data season-wise. This graph clearly showed the highest polyphenolic content in samples collected during the summer ($p < 0.05$). Although, for each of the extraction systems, non-significant change has been recorded between samples collected in winter and monsoon season.

In Sec-3, samples of six different groups of individual solvent systems were compared for their total polyphenolic content. For this analysis, samples from all six groups were calculated and compared. This calculation would provide us with clarity about the change in polyphenolic content in plant samples collected in the same season but in different years. As seen in Fig. 3, for methanolic extracts, no considerable difference was seen among samples of Groups 1 and 4, Groups 2 and 5, and similarly between Groups 3 and 6. Hence, for this extract, samples collected in the same seasons of different years showed no change in polyphenolic content. Similar results were reported for ethanolic, aqueous, acetone, and benzene extracts (Figs. 4-8). For chloroform extracts, samples collected in the summer and monsoon

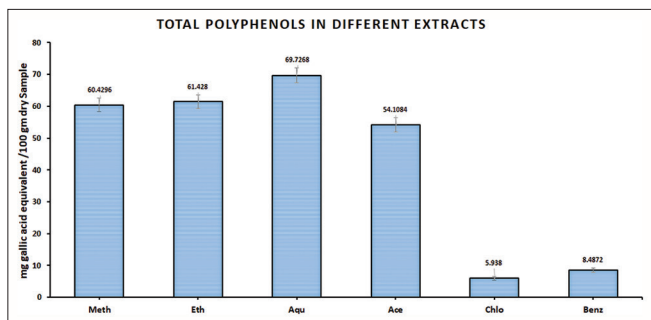


Fig. 1: Comparative study of polarity and nature of solvent on the extraction of total polyphenolic content. Values are expressed in mean \pm SE (n=30 for each bar)

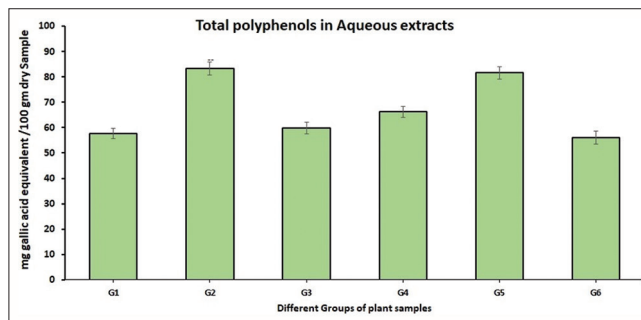


Fig. 5: Comparative study of aqueous extracts of six groups for their total polyphenolic content. Values are expressed in mean \pm SE (n=5 for each bar)

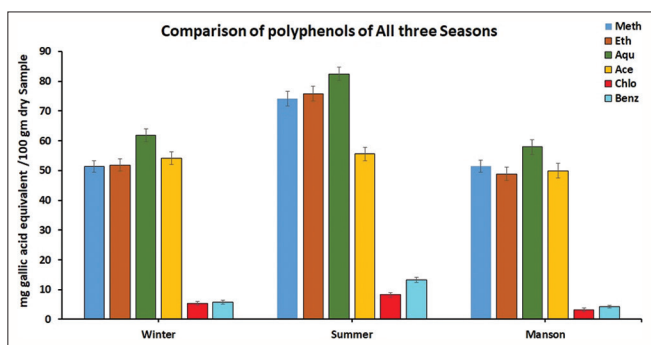


Fig. 2: Comparative study of the impact of the season (i.e., winter, summer, and Mansoon) on total polyphenolic content. Values are expressed in mean \pm SE (n=10 for each bar)

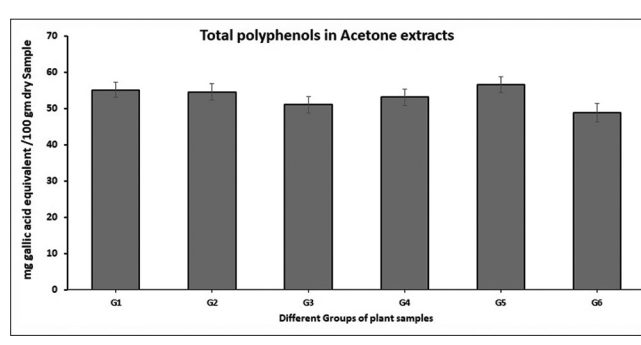


Fig. 6: Comparative study of acetone extracts of six groups for their total polyphenolic content. Values are expressed in mean \pm SE (n=5 for each bar)

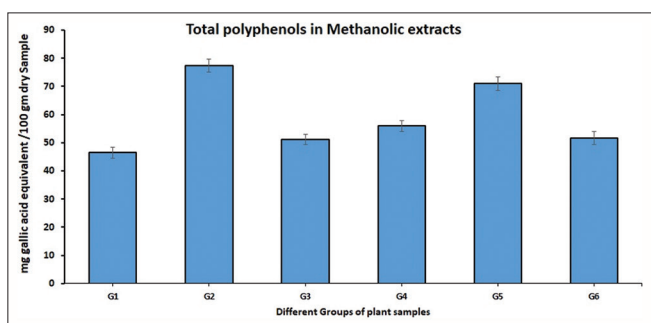


Fig. 3: Comparative study of methanol extracts of six groups for their total polyphenolic content. Values are expressed in mean \pm SE (n=5 for each bar)

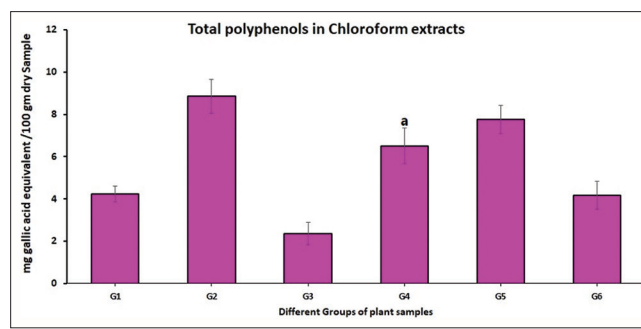


Fig. 7: Comparative study of chloroform extracts of six groups for their total polyphenolic content. Values are expressed in mean \pm SE (n=5 for each bar)

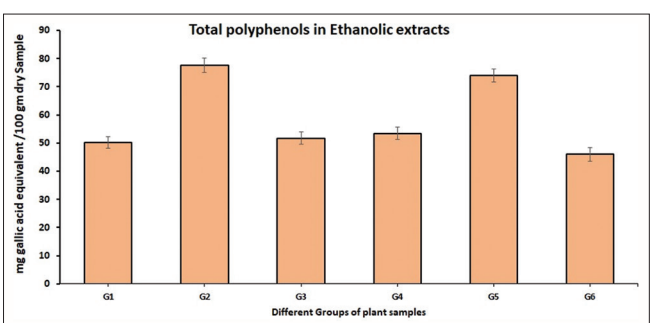


Fig. 4: Comparative study of ethanol extracts of six groups for their total polyphenolic content. Values are expressed in mean \pm SE (n=5 for each bar)

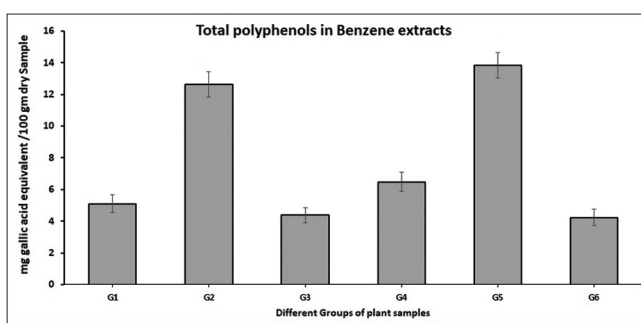


Fig. 8: Comparative study of benzene extracts of six groups for their total polyphenolic content. Values are expressed in mean \pm SE (n=5 for each bar)

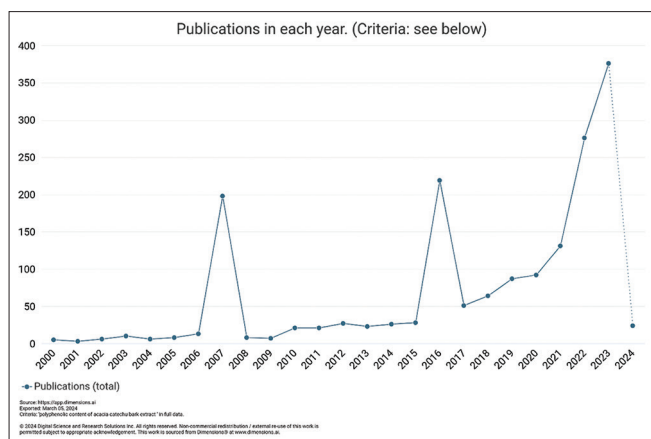


Fig. 9: Database showing number of publications from 2000 to 2024 on "polyphenolic content of *Acacia catechu* bark extract"

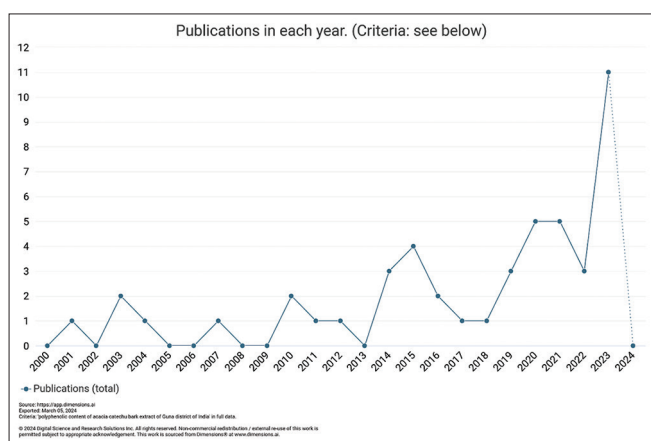


Fig. 10: Database showing a number of publications from 2000 to 2024 on "polyphenolic content of *Acacia catechu* bark extract of Guna district of India"

seasons of different years exhibited no difference in polyphenolic content, but in the case of samples collected in the winter season, the sample collected in 2017 had greater polyphenolic content ($p < 0.05$) than the sample collected in 2016. In addition, the similar polyphenolic content in acetone extracts in almost all groups of samples has also put a different pattern than other extracts. It can be said that except for acetone, all other solvent systems, that is, methanol, ethanol, aqueous, chloroform, and benzene, demonstrated linearity among the findings.

The above-mentioned figures depicted the presence of considerable amount of polyphenolic compounds in test samples. Moreover, aqueous extracts of samples collected in the summer season can be taken as most appropriate for further investigation. Although, methanolic and ethanolic extracts were also seen to exhibit considerable amount of polyphenolic compounds.

The bibliographic database of past 50 years was analyzed (but to remove complexity, only the database of past 25 years is depicted in Figs. 9 and 10). This indicated total number of publications on the studied parameter is limited. The total number of publications on "polyphenolic content of *A. catechu* bark extract" from 1975 to until now is 1,867, this includes 449 articles, 926 book chapters, and 325 edited books. The data search using keywords "polyphenolic content of *A. catechu* bark extract of Guna district of India" from 1975 to until now are showed total of 47 results, including only 2 research articles and 38 references of edited Book. These finding clearly showed that the researches on the same particularly in Guna region is meager.

DISCUSSION

Numerous intervention studies, *in vitro* mechanistic information, and epidemiological research support the role that polyphenols play in protecting against chronic disorders [2,5]. Almost all the *Acacia* plants have been recognized for the presence of significant amount of phenolic compounds as has also seen in the present study. For example, in pods, leaves, stems, barks, flowers, and roots of *Acacia farnesiana*, commonly named as sweet *Acacia* significant amount of polyphenolic compounds have been reported. *A. farnesiana* pods in particular have been recognized as a source of phytochemicals with potent antibacterial and preventive activities against oxidative stress [7,10,16,17].

According to some researches, the common dietary sources of phenolic compounds are fruits, vegetables, and drinks [2,18]. The main sources of phenolic compounds are strawberries and their derivatives, such as juices, as well as commonly eaten fresh and processed fruits such as raspberries, cranberries, apples, grapes, pears, and jams [3,5,18,19]. Flavanols reduce endothelial dysfunction, decrease blood pressure and cholesterol, and regulate energy metabolism. It has been demonstrated that the polyphenols of coffee and tea can lessen the chance of acquiring type 2 diabetes [3]. Polyphenols are found in tea, cocoa, fruits, and vegetables and have the ability to impact human health in a favorable way. Cocoa flavan-3-ols have been linked to a reduced incidence of heart attack, stroke, and diabetes [2,18]. Polyphenols are recognized for their ability to influence the makeup of the gut microbiota in a manner that promotes improved human health. The gut microbiota transforms polyphenols into bioactive molecules with medicinal properties [19].

Earlier researchers have found that polyphenolic chemicals, tannins, flavonoids, and carbohydrates were detected in the aqueous, ethyl acetate, and N-butanol fractions of *Acacia nilotica* pod extracts. The residual fraction included only tannins and carbohydrates. The extracts did not include anthraquinones, alkaloids, terpenes, or steroids [20]. Both plants' petroleum ether and ethyl acetate extracts exhibited superior antibacterial activity in comparison to the methanol extracts. Both plants' ethyl acetate extracts showed superior antioxidant and cytotoxic effects compared to the petroleum ether and methanol extracts [9,16,21,22]. These solvent-specific protective activities might be observed because the different phenolic compounds show differential solubilities in different solvents. In the present study, also similar patterns have been seen, where the polar solvents showed greater solubility of the test samples than non-polar solvents.

Many studies have revealed the benefits of phenolic compounds, including their anti-aging, anti-inflammatory, antioxidant, and antiproliferative properties [1,22]. In addition to the aforementioned adjustments, antioxidant enzymes are necessary to combat oxidants. The crucial feature of suppressing glucosidase and amylase, which are vital enzymes and responsible for the digestion of dietary carbohydrates to glucose, is possessed by polyphenols, particularly flavonoids, phenolic acids, and tannins [12,15,20,23]. Multiple *in vivo* and *in vitro* studies have been carried out to assess their health effects. Polyphenols have a crucial role in protecting the body from environmental stressors and neutralizing reactive oxygen species, which may cause various diseases [24]. Although research has been thorough, the precise ways in which polyphenols work in the human body have not been definitively confirmed. However, there is compelling evidence indicating that certain targets including nitric oxide metabolism, carbohydrate digestion, and oxidative enzymes play a crucial role in providing health advantages [21,23-26].

Similarly, some have mentioned the anticancer and apoptotic effects of methanolic extract of *A. catechu* heartwood on the human breast adenocarcinoma cell line. The extract displayed considerable cytotoxicity against cultivated MCF7 cells and triggered apoptosis, as shown by flow cytometric analysis and morphological examination. Immunoblot research indicated that the extract induced apoptosis by increasing the Bax/Bcl2 ratio, activating the caspase cascade, and

ultimately cleaving poly adenosine ribose polymerase [27,28]. In another study, the methanolic extract of the heartwood of *A. catechu* was seen to exhibit DNA protective, iron chelating, and antioxidant activities along with the antiradical action against superoxide, nitric oxide, hydrogen peroxide, and hypochlorous acid radicals [29].

The catechin molecule is classified as a flavan-3-ol, which is a sort of secondary metabolite that has an antioxidant function in plants. It is classified as a flavonoid, which is a subgroup of the polyphenolic family [30]. The name of the chemical class known as catechins comes from the word "catechu," which refers to the tannic juice or the boiling extract of the *A. catechu* plant. The catechins, epicatechin, epicatechin-3-O-gallate, and epigallocatechin-3-O-gallate are the most abundant in *A. catechu* [28]. Epigallocatechin gallate compound (EGCG), is actually epigallocatechin-3-gallate, that formed by esterification of epigallocatechin and gallic acid under certain specific metabolic pathway. The catechin known as EGCG, which is found in tea in the highest concentration, is a kind of polyphenol that is now being investigated for its possible impact on human health and illness. Numerous dietary supplements include EGCG as an active ingredient [20,29,31-33].

According to recent findings, dietary plant polyphenols and polyphenol-rich foods affect how carbohydrates and lipids are metabolized [34]. They also reduce hyperglycemia, hyperlipidemia, blood pressure, and insulin resistance. Some scientific data stated that polyphenolic compounds also enhance cell function, stimulate insulin secretion, improve the metabolism of adipose tissue, and reduce inflammation, as also seen by other [2,5,23,35,36]. Long-term consequences of diabetes, such as cardiovascular disease, neuropathy, nephropathy, and retinopathy, may also be prevented by polyphenolic substances. For example, crimson fruit of *Viburnum dilatatum* containing polyphenolic substances has potent antioxidant properties [37-40]. The polyphenolic compounds of *A. catechu* plant have also been utilized for skin treatment [41-42]. The valuable functions suggested that unutilized vegetation should be further investigated to meet the needs of the local people; this could also be a better substitution of costly allopathic supplements.

CONCLUSION

Non-communicable diseases are becoming more common worldwide due to a sedentary lifestyle and many factors such as smoking and the intake of unhealthy food. Local natural supplements may be effective in addressing health conditions. Exploring novel, cost-effective, and easily available sources of polyphenolic compounds from natural sources might be a valuable treatment. Our objective was to compile scientific data on the polyphenolic content found in the native flora of Guna region. The current discovery yields favorable outcomes in this regard; nonetheless, these data should serve just as foundational findings for future study. Additional research is required to uncover any potential therapeutic benefits of the substance.

AUTHORS CONTRIBUTION

I, Mrs. Archana Tiwari, assistant professor, Government P.G. College Guna, District Guna, Madhya Pradesh, India, has done the above complete research work under the guidance and supervision of Professor (Dr.) Avinash Tiwari, Vice chancellor and Professor, School of studies in Botany, Jiwaji University, Gwalior, (M.P.) India, under whom I am pursuing my present research work as Ph.D. candidate.

CONFLICTS OF INTERESTS

No conflict.

SOURCE OF FUNDING

Nil.

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