WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 12, Issue 20, 937-946. Research Article ISSN 2277–7105

QUALITATIVE ANALYSIS OF TANNINS IN DIFFERENT BARK EXTRACTS OF *ACACIA CATECHU* COLLECTED FROM GUNA DISTRICT, MADHYA PRADESH, INDIA

Archana Tiwari[1]* and Prof. Dr. Avinash Tiwari[2]

[1]Assistant Professor, Department of Botany, Government P.G. College Guna (M.P.) 473001, India.

[2]Vice Chancellor, (Professor, School of Studies in Botany), Jiwaji University, Gwalior, (M.P.) 474011, India.

Article Received on
29 September 2023,

Revised on 19 Oct. 2023,
Accepted on 08 Nov. 2023

DOI: 10.20959/wjpr202320-30298

***Corresponding Author**
Archana Tiwari
Assistant Professor,
Department of Botany,
Government P.G. College
Guna (M.P.) 473001, India.

ABSTRACT

Many plant species include tannins, which are water-soluble polyphenolic macromolecules that act as a defense against predators and may also help regulate plant growth. The purpose of this research was to assess the tannin content of several test extracts from test plant samples. Thirty separate samples of *Acacia catechu* bark were gathered for this purpose from Guna District, Madhya Pradesh, India, during various seasons. Six extracts were then prepared from each sample. After that, several techniques were used to determine if tannins were present in these extracts or not. The findings demonstrated that methanol, aqueous, and sometimes acetone extracts had a considerable quantity of tannin content; ethanol extracts, on the other hand, had a much lower tannin content, and both chloroform and benzene extracts were found to be free of the same. This exploratory study might serve as a foundation for further investigations on the best solvent to use for extracting tannin compounds from the materials under review.

KEYWORDS: Tannins, *Acacia catechu* bark extract, qualitative analysis, solvent medium, seasonal effects.

INTRODUCTION

Tannins are a group of polyphenolic biomolecules that possess astringent properties. They have the ability to connect with proteins, as well as other organic substances such as amino acids and alkaloids, resulting in their precipitation.[1] These compounds are found in a variety

of plant species and serve as a defense mechanism against predation, perhaps aiding in the regulation of plant development.[2] According to previous studies[1,2], it has been shown that these factors have been associated with a reduction in feed intake, growth rate, feed efficiency, net metabolizable energy, and protein digestibility in experimental animals. Hence, it may be inferred that dietary items that contain high levels of tannins are regarded as having little nutritional benefits. Nevertheless, current research suggests that the primary impact of tannins is not attributed to their ability to restrict food ingestion or digestion, but rather to their capacity to reduce the efficiency of converting ingested nutrients into new bodily components.[3]

Several studies have shown that certain tannin compounds possess the ability to decrease the mutagenic activity of various mutagens.[4] Numerous carcinogens and/or mutagens generate oxygen-free radicals that engage in interactions with cellular macromolecules.[3] The possible anticarcinogenic and antimutagenic effects of tannins may be attributed to their antioxidative properties, which play a crucial role in safeguarding against cellular oxidative damage, such as lipid peroxidation. Tannins and similar substances have been shown to have inhibitory effects on the production of superoxide radicals.[5]

In the culinary and medicinal industries, tannins' antioxidant qualities are frequently used. Numerous investigations have been carried out in the last few years to determine tannins' pertinent antioxidant activity.[4,6] Due to their ability to serve as antioxidants, which may help prevent cancer, heart disease, and osteoporosis, tannins have gained a lot of attention.[5,6] Tannins are naturally occurring polyphenolic chemicals with antimicrobial properties. Studies have looked at how various tannin concentrations affect an animal's ability to develop, its gut microbes, and its morphology. According to some studies, tannins may shield against viruses including norovirus, HIV, and bovine adeno-associated virus.[7] According to some recent research, plant tannins reduce inflammation by blocking prostaglandin-E2 and NO. The majority of tannins that are derived from various plants have anti-inflammatory properties.[8] Furthermore, many additional health-promoting and therapeutic properties of tannins derived from plants have been investigated.[3,6-8]

A well-known and often utilized plant in traditional Indian medicine is *Acacia catechu*.[9] This plant's heartwood is highly valued in India where it is used to make the commercially important goods cutch and katha.[10] The ingredient katha (a tannin compound) is used in South Asian paan, which is often chewed and swallowed after meals. It is also used to

beverages, sweets, sauces, and ice cream as a flavouring element.[9,10] Cutch is used to modify the viscosity of drilling mud in oil-well drilling as well as a tanning agent for leather, dye, mail bags, and other materials.[11]

Healing wounds with *Acacia catechu* bark is a useful medicinal practice. The bark extract has been shown to have astringent qualities in a number of experimental animals.[9,11] From a dentistry standpoint, it treats bleeding gums when applied topically as a powder. When used as a gargle to treat sore throats, poor breath, and tooth decay, it is highly helpful.[12] Additionally, this plant produces and secretes a wide range of active secondary metabolites, most of which are phenolic compounds with potent insecticidal and antibacterial properties.[13] These materials are oils that are vital. Many medications, complementary therapies, and natural remedies often employ the same because of this potential.[12] Numerous phytochemicals, including resins, tannins, saponins, flavonoids, alkaloids, and glycosides, were confirmed to be present in *Acacia catechu* by biochemical research.[10,13] This plant has also been shown to contain the methyl ester of caprylic acid, lauric acid, myristic acid, and 2-Ethyl-3-methyl-1-butene.[10]

It is widely distributed geographically among several Madhya Pradesh districts, including Rajgarh, Dhar, Guna, Ashoknagar, Barwani, Khargone, Harda, and Chhatarpur. The Guna location was chosen for our current study project due to the population's high tree density.[12] Comparative screening of tannin compounds in different extracts of test substances in a concentration-dependent manner with regard to the parameter under inquiry was the main goal of this study. Moreover, seasonal differences have been shown to affect plant phytochemistry. Thus, samples collected in two successive years during different seasons were also analyzed using the same parameters.

Morphology of the *Acacia catechu* plant

Acacia catechu, a deciduous tree of the Fabaceae family, is extensively planted. The tree grows 9–12 meters tall, but its blossoms are pale-yellow. The botanical specimen has cylindrical spikes that produce flat, rectangular fruits.[8] Short, curving spines and bipinnately complex leaves with 50 pairs of feather-like leaflets characterize the tree. Bark on stems ranges from brown to grey.[11] Small, bipinnately complex foliage measures two to six millimeters. Pairs of pinnae define its arrangement. A glandular rachis connects leaf pairs.[12] In winter, inland plants had cylindrical inflorescences and axillary pedunculate spikes.

Blooms are actinomorphic or zygomorphic and 5–10 centimeters long. The sessile organisms are pentamerous and creamy white to light yellow.[9-11]

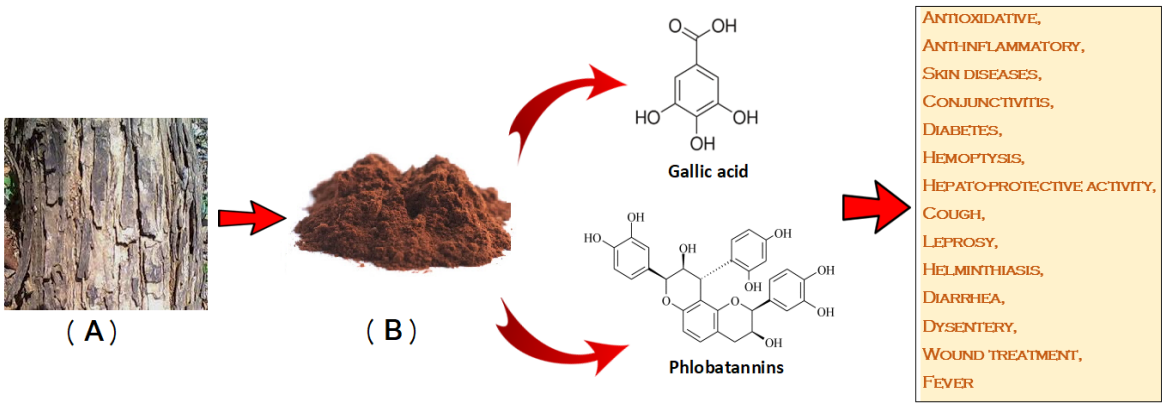

Fig. 1: Schematic representation of (A) Acacia catechu stem, (B) Powdered Tannin with phyto-active components and their medicinal properties.

MATERIALS AND METHODS

Chemicals

Gelatin, Sodium nitroprusside, Phosphoric acid, Toluene, Ethyl acetate, n-Hexane, Ethyl Ether, Ethanol, benzene, methanol, were supplied by Hi Media Laboratories Ltd., Mumbai, India. Ferrous sulphate (FeSO4), Sodium chloride (NaCl), sodium hydroxide (NaOH), Sodium nitrate (NaNO3), Ferric chloride (FeCl3) and Potassium ferricyanide, and all other reagents were purchased from Sisco Research Laboratories (SRL) Pvt. Ltd and from E-Merck (India) Ltd., Mumbai, India.

Collection and processing of bark samples

Acacia catechu bark was randomly gathered from Biloniya village, Guna (Madhya Pradesh) trees. A one-km circular was collected. Bark was always chosen 1.3 meters above ground level to preserve uniformity.[11] The weight of evenly round or unbroken bark samples was measured using a portable digital scale after hand cleaning. Five plant samples were taken in mid-January, mid-May, and mid-September throughout the winter, summer, and rainy seasons. The data collection procedure lasted two years, 2016 and 2017. After desiccation in a darkened region, the materials were mechanically ground at room temperature and filtered through a 0.5-mm fine mesh in a controlled laboratory setting. After that, powdered samples were kept at 4°C.[12,13]

Bark extract Preparation

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.[8, 11-13]

Qualitative analysis of Tannins

(a) $FeCl_3$ test: 100 µl of alcoholic $FeCl_3$ reagent was added to 2 ml of sample extract for this test. The presence of tannins was revealed by the emergence of blue colour.

(b) Gelatin's test: To do this, put 1 ml of sample extract and 1 ml of 1% gelatin solution that has been saturated with sodium chloride into a test tube and mix. The sample included tannins as shown by the formation of white precipitate.

RESULTS AND DISCUSSION

The sign "+" meant that the phytochemicals that were tested were present. There was a fairly high concentration of phytochemicals when the '++' sign was used. There was a much higher concentration of phytochemicals when the '+++' sign was used. Table 1 shows $FeCl_3$ qualitative tannin testing. Most examined chloroform and benzene extracts were tannin-free. This may be because tannins are insoluble in non-polar solvents like chloroform and benzene. Other extracts including methanolic, ethanolic, aqueous, and acetone are superior solvents. In all samples, methanolic and aqueous solvents had more tannins than ethanolic and acetone extracts. No seasonal fluctuations in tannin concentration were seen in these samples (Table 2). Qualitative tannin assays employing Gelatin's test technique in various sample extracts provide comparable findings. Most examined chloroform and benzene samples are tannin-free using this approach. Verified that chloroform and benzene do not dissolve tannins. Other extracts including methanolic, ethanolic, aqueous, and acetone work well as solvents. In the tannin concentration experiment, methanolic, ethanolic, aqueous, and acetone extracts had

similar colour intensity. Summer samples from 2016 and 2017 have more tannins than winter and rainy season samples (table 2).

Table 1: Qualitative tests for Tannins using $FeCl_3$ test method in different extracts of samples.

Test for Tannins: $FeCl_3$ 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 2: Qualitative tests for Tannins using Gelatin's test method in different extracts of samples.

Test for Tannins: Gelatin'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.

The results revealed that the tested bark samples of bark of *Acacia catechu* collected from Guna district are good source of alkaloids, flavonoids, and tannins, etc. The presence of above-mentioned compounds is seen to be more or less co-related with the season.

As mentioned earlier also, different parts of *Acacia catechu*, served as excellent source medicinal bioactive components with several uses.[14] Some researchers have found that biochemical analysis of *Acacia catechu* verified the presence of tannin compounds with other phytoactive components, as also seen in the present investigation.[12,15] The many parts of plant, including heartwood, bark, seeds and leaves, each have unique pharmacological qualities that may be used to cure a variety of illnesses.[11,14,16] Several pharmacological activities, including anti-inflammatory, antioxidant, antibacterial, antipyretic, hypoglycemic, and hepatoprotective characteristics, are shown by Acacia catechu extracts have been reported to co-related with the tannin content of the same.[10-13,16]

Tannins are important to medicinal bio-molecules and have been reported for their beneficial health effects, for example, a tannin substance found in *Hamamelis virginiana* is utilized for treatment of skin problems.[2,4] For decades, numerous studies have demonstrated the antioxidant activity of tannins. The culinary and medicinal industries make extensive use of tannins for their antioxidant qualities.[2,4-6] Tannins have received a lot of attention due to protecting efficacy from osteoporosis, cancer, pain, inflammation and cardiovascular disease. For intense, tannin from grape seed extracts has been reported to decrease total and bad cholesterol from blood.[3,5,17]

Some polyphenolic tannins have been recognized for their inherent antibacterial properties. Additionally, it has been noted that tannins alter the quantity of microorganisms in chickens' small intestine and colon.[18] According to certain additional research, tannins may protect against viral infections including HIV, bovine adeno-associated virus, and norovirus. In addition to this, most tannins that are derived from various plants have anti-inflammatory properties. For example, grape seed procyanidin extract was used in an in vitro experiment with obese Zucker rats to show that it may inhibit inflammation brought on by obesity by regulating the production of cytokines.[2-5,7,18]

CONCLUSION

As a natural polyphenolic substance, plant tannins have been found to have a variety of biological functions. In addition, different extraction medium and season of sample collection

can affect the tannin content of *Acacia catechu* bark extracts. However, the physiological effects of the same are not clear but on the basis of available data, this can be predicted as potential source of therapeutic agent. More studies needed to be done to understand the quantity, structure, and other characteristics of the same.

ACKNOWLEDGEMENTS

I express my sincere thanks to Dr. Sangeeta Sharma, Guest Faculty, School of Studies in Botany, Jiwaji University, Gwalior, (M.P.) who helped in data analysis.

REFERENCES

1. Chung KT, Wong TY, Wei CI, Huang YW, Lin Y. Tannins and human health: a review. Crit Rev Food Sci Nutr, 1998 Aug; 38(6): 421-64.
2. Ogawa S, Yazaki Y. Tannins from *Acacia mearnsii* De Wild. Bark: Tannin Determination and Biological Activities. Molecules, 2018; 23(4): 837.
3. Choubey S, Goyal S, Varughese LR, Kumar V, Sharma AK, Beniwal V. Probing Gallic Acid for Its Broad-Spectrum Applications. Mini Rev Med Chem, 2018; 18(15): 1283-1293.
4. Weisburger JH. Antimutagens, anticarcinogens, and effective worldwide cancer prevention. J Environ Pathol Toxicol Oncol, 1999; 18(2): 85-93.
5. Yang B, Liu P. Composition and biological activities of hydrolyzable tannins of fruits of *Phyllanthus emblica*. J Agric Food Chem, 2014; 62(3): 529-41.
6. Wang J, Sheng Z, Liu Y, Chen X, Wang S, Yang H. Combined proteomic and transcriptomic analysis of the antimicrobial mechanism of tannic acid against *Staphylococcus aureus*. Front Pharmacol, 2023; 14: 1178177.
7. Falcao L, Araújo MEM. Vegetable Tannins Used in the Manufacture of Historic Leathers. Molecules, 2018; 23(5): 1081.
8. Sargsyan L, Vill V, Hippe T. Investigations of vegetable tannins as hair dyes and their interactions with pre-bleached hair fibres. Int J Cosmet Sci, 2020; 42(4): 320-327.
9. Reddy MBK, Gowda KPS, Arora AK, Study of wound healing activity of aqueous and alcoholic bark extracts of *Acacia catechu* on rats. J Pharmac Sci, 2011; 1(3): 220-5.
10. Ray D, Sharatchandra KH, Thokchom IS. Antipyretic, antidiarrhoeal, hypoglycaemic and hepatoprotective activities of ethyl acetate extract of *Acacia catechu Wild*. in albino rats. Indian J Pharmacol, 2006; 38(6): 408-13.

11. Hazra B, Sarkar R, Ghate NB, Chaudhuri D, Mandal N. Study of the protective effects of Katha (heartwood extract of *Acacia catechu*) in liver damage induced by iron overload. J Environ Pathol Toxicol Oncol, 2013; 32(3): 229-40.
12. Baluja S, Chanda S, Solanki A, Kachhadia N. Phytochemical studies of *Acacia catechu*. Indonesian J Pharmacy, 2012; 23(4): 238-47.
13. Joshi S, Subedi YP, Paudel SK. Antibacterial and antifungal activity of heartwood of *Acacia catechu* of Nepal. J Nepal Chem Society, 2011; 27: 94-9.
14. Baranitharan M, Alarifi S, Alkahtani S, Ali D, Elumalai K, Pandiyan J, Krishnappa K, Rajeswary M, Govindarajan M. Phytochemical analysis and fabrication of silver nanoparticles using *Acacia catechu*: An efficacious and ecofriendly control tool against selected polyphagous insect pests. Saudi J Biol Sci, 2021; 28(1): 148–156.
15. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: A review. Int Pharm Sci, 2011; 1: 98–106.
16. Baluja S, Chanda S, Solanki A, Kachhadia N. Phytochemical studies of *Acacia catechu*. Indonesian J Pharmacy, 2012; 23(4): 238-47.
17. Tiwari A, Tiwari A. Amazing Antimicrobial and Wound Healing Potential of *Acacia Catechu* Bark Extracts-A Review. Asian J Pharm Clin Res, 2021; 4(10): 1-7.
18. Li XC, Liu C, Yang LX, Chen RY. Phenolic compounds from the aqueous extract of *Acacia catechu*. J Asian Natural Products Res, 2011; 13(9): 826-30.