ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 17, Issue 5, 2024

Online - 2455-3891
Print - 0974-2441

Research Article

EVALUATION OF COMPARATIVE *IN VITRO* FREE RADICAL SCAVENGING POTENTIAL OF BARK EXTRACTS OF *ACACIA CATECHU*

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Received: **29 October 2023,** ***Revised*** **and** ***Accepted: 25 January 2024***

ABSTRACT

Objective: This study was aimed at investigating the comparative 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging potential of different bark extracts of *Acacia catechu*. The assessment was conducted following concentration-dependent and seasonal-dependent impacts of the same using samples collected in different seasons over 2 successive years.

Methods: In this, six extracts using ethanol, methanol, aqueous, acetone, chloroform, and benzene solvents were prepared. For the *in vitro* study, a standard DPPH solution (0.15 mM) was used to check how well the test samples got rid of free radicals. The major biochemical components of test plants, such as quercetin, gallic acid, and catechin, were used as standard drugs.

Results: Among all test drug concentrations, 31.5–500 µg/mL drug concentrations were observed to be effective, whereas 15.25, 750, and 1000 µg/mL concentrations exerted negligible scavenging effects, and 125 µg/mL concentrations were found to be most effective ($p<0.01$ or more). The order of scavenging potential of different extracts is seen to be methanolic≥ethanolic>aqueous>acetone>chloroform>benzene. The samples collected during the rainy season were the least effective. Samples collected during the winter and summer seasons, on the other hand, were both more effective ($p<0.05$) at removing DPPH free radicals.

Conclusion: This study helps to provide primary data on the concentration range, impact of the extraction medium, and sample collected in different seasons. Probably, these findings signify a notable progression in the investigation of the utilization of native plant species for medicinal purposes.

Keywords: *Acacia catechu* bark extracts, DPPH, Quercetin, Gallic acid, Antioxidant assay, Free radical scavenging.

 DOI: http://dx.doi.org/10.22159/ajpcr.2024v17i5.49735. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Various free radicals are generated endogenously in the body as byproducts of metabolic processes [1]. In limited numbers, these are beneficial and serve as active agents in a number of protective immunological pathways [2,3]. Occasionally, these play specific roles in assisting the immune system in identifying and eliminating infections by activating a process referred to as mitohormesis [4]. The potential impact of short-term oxidative stress on the mitigation of aging and other metabolic disorders cannot be overlooked [2-4]. The overproduction of the same has already been known to lead to several severe impairments such as DNA mutation, DNA fragmentation, DNA damage, lipid peroxidation, protein modification, apoptosis, modification of gene expression, and membrane damage via oxidative stress [1,5,6], Thus, oxidative stress is a state of the body in which the body loses its ability to eliminate reactive free radicals and is unable to avoid harmful effects caused by them [3,7].

However, almost all types of metabolic disorders, including cancer, diabetes, hormonal imbalance, cardiac problems, obesity, Parkinson's disease, Alzheimer's disease, etc., have been scientifically proven to be mediated through oxidative stress [1,5,8]. Hence, there is an urgent surge in interest in the use of naturally available plant-based antioxidants as a means to combat the detrimental effects of free radicals [3].

Numerous studies have shown the existence of inherent antioxidants in both edible and medicinal plant species [9,10]. Naturally occurring antioxidants, including polyphenols, flavonoids, tannins, vitamins, and carotenoids, possess a diverse range of therapeutic properties, such as anti-inflammatory, antibacterial, antiviral, anti-aging, wound healing, anticancer effects, etc. [4,9-11]. In many studies, plant-based pharmaceuticals have been recognized to consist of intricate mixtures of protective bioactive substances [3,9,12,13]. Although the use of herbal remedies is widely used throughout the world [3]. In almost all developing nations, including India, people mostly rely on local traditional medicine, which often includes herbalism, as their primary form of health care [14]. Notably, over 30% of pharmaceutical preparations continue to be derived either directly or indirectly from botanical sources [5,15].

The traditional medicinal practices of India have engrossed individuals by virtue of their enduring cultural significance and intergenerational transmission [14]. Moreover, the traditional system of herbal medicines for the prevention and treatment of various diseases is observed by over 80% of the people residing in the state of Madhya Pradesh, India [16-18]. The aforementioned methods provide many advantages, such as their convenient availability, extended shelf life, and ease of transportation [4,6]. In addition to this, the cost disparity between contemporary drugs and traditional herbal therapies often renders the latter a cost-effective choice with a lower incidence of adverse effects [1,5].

Acacia catechu is distributed across India, with the exception of cold regions. The scientific position is given in Table 1 [19,20]. Because of favorable atmospheric conditions, this particular plant is widely distributed over many districts of Madhya Pradesh, with a more notable presence in Rajgarh, Dhar, Guna, Ashoknagar, Barwani, Khargone, Harda, and Chhatarpur [19-23]. This specific species was chosen for our study because of its significant abundance in the Guna region and the lack of published scientific research on it [24,25].

Table 1: Scientific classification of Acacia catechu ((L.f.) P.J.H.Hurter and Mabb.)

Kingdom	Plantae
Phylum	Tracheophyta
Clade	Angiosperms
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Fabales
Family	Fabaceae
Genus	Acacia Mill.
Species	*Acacia catechu* wild-black cutch

Multiple available pieces of literature have revealed the use of the A. catechu tree by Ayurvedic practitioners for several years in the management and prevention of a diverse array of health conditions [5,12,24,25]. The plant is also referred to as Khadira, kattha, or black cutch [13]. Studies undertaken by the pharmaceutical sector have demonstrated that the phytochemicals of this plant have disease-prevention potential [26]. For example, catechin, a bioactive compound abundantly present in this plant, has antioxidant properties and also acts as an antibacterial agent [27]. Tannin components of the same have been recognized for their potential therapeutic efficacy in wound healing [28].

The bark of A. catechu contains various bioactive compounds, including catechin, epicatechin, acacatechin, kaempferol, quercetin, quercitrin, tannins, phlobatannin, ascorbic acid, riboflavin, thiamine, niacin, rutin, isorhamnetin, porifera steroyl acyl glucosides, gallic acid, and carotenoids, which belong to a group of antioxidants [29-31]. The structures of a few bioactive components have been depicted in Fig. 1. Some scientific data also revealed the anti-oxidative and free radical-scavenging potential of the same [32]. Although some separate reports have shown the medicinal properties of methanolic, ethanolic, and aqueous bark extracts of A. catechu plants from different regions, scientific data on the same from the Guna region are meager [24]. In addition, none of the available data have explained the comparative antioxidative activity of six different extracts of the same, prepared using solvents of different polarities.

The main aim of this study was to investigate the relative antioxidative potential of different extracts in a concentration-dependent manner on the studied parameter. Moreover, seasonal changes have also been documented to affect the phytochemistry of plants [33]; hence, samples collected in different seasons of 2 successive years were also studied using the same parameter. Since *in vitro* studies provide a simplified model for investigation, the outcomes may be indicative of effects in *in vivo* systems [1,6,7]. Therefore, present *in vitro* research has been conducted as an initial phase of the study.

METHODS

Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH), gallic acid, quercetin dihydrate, L-ascorbic acid, and catechin were purchased from Sigma-Aldrich, St. Louis, MO, USA. While ethyl acetate, n-hexane, ethyl ether, ethanol, benzene, methanol, and sulfuric acid were supplied by Hi Media Laboratories Ltd., Mumbai, India, chloroform, glacial acetic acid, double distilled water (DDW), and all other reagents were purchased from Sisco Research Laboratories Pvt. Ltd. and E-Merck (India) Ltd., Mumbai, India.

Collection and processing of bark samples

The bark samples of the plant (specimen deposited in the herbarium of Jiwaji University Gwalior, MP, with voucher number AC-101A-101O/SOB2016 and AC-102A-102O/SOB2017) were collected randomly from trees of A. catechu at Biloni village, Guna (MP), covering a diameter of 1 km. To maintain homogeneity, the bark was picked out at (Diameter at Breast Height; 1.3 m above the ground). About 50 mm of circular or healthy bark was collected, manually cleaned, and weighed using a portable digital balance with an accuracy of 0.001 g [31]. Samples were collected from five plants in each season, such as winter (in mid-January), summer (in mid-May), and a rainy season (in mid-September), over a duration of 2 successive years, namely 2016 (considered groups 1, 2, 3 for the respective above-mentioned seasons) and 2017 (considered groups 4, 5, 6 for the respective above-mentioned seasons). Under laboratory conditions, shade-dried samples were powdered using a mechanical grinder at room temperature and strained through a fine mesh (0.5 mm). These powdered samples were then stored at 4°C [31,32].

Preparation of various bark extracts

Various extracts of the test sample were prepared using standardized protocols. For the preparation of the aqueous extract, 50 g of bark powder was extracted with 1000 mL of double-distilled water at room temperature with continuous stirring by a magnetic stirrer for 3 h and then left for 24 h [31]. The filtrate was then dried and weighed. Similarly, for the preparation of extracts of organic solvents (namely 80% ethanol, methanol, benzene, chloroform, and acetone), 50 g of dried fine powder from the samples was thoroughly mixed with 1000 mL of volume at room temperature. Following the same procedure as mentioned above, all extracts were prepared, and then, dried extracts were stored in the refrigerator at 4°C. At the time of the experiment, stock extracts were prepared by dissolving 1000 µg/mL dried extracts in DDW for further experimentation [29,31,32].

Experimental design

DPPH is a synthetic, stable nitrogen-free radical. In this assay system, an antioxidant/bioactive component of the extract donates an electron to the free radical (i.e., DPPH reduced by antioxidants), which results in the change in color of the assay mixture. This change is read by a UV-VIS spectrophotometer [1]. For this, 0.5 ml of a methanolic stock solution of DPPH (0.15 mM) was added to 1 ml of sample extract and then incubated for 30 min in the dark at 20°C. A control tube was prepared by adding methanol in place of the extract. Since quercetin, gallic acid, and catechin are reported bioactive ingredients of test bark, they are hence used as standard drugs [26,29]. As no prior scientific data were available on the *in vitro* study of test extracts, a wide range of concentrations (15.25, 31.5, 62.5, 125, 250, 500, 750, 1000 µg/mL) of bark extracts were tested and standardized. Optical density was taken at 517 nm, and percent (%) scavenging activity was determined. The scavenging activity of the test drugs was expressed following the given formula, as done earlier.

$$\%\,Inhibition\,(\%\,Scavenging) = \frac{(control\ OD - sample\ OD)}{control\ OD} \times 100$$

Statistical analysis

Data were expressed as mean±SE. Statistical analysis was done considering one-way analysis of variance followed by an unpaired Student's t-test using a trial version of Prism 9 software for Windows (Graph Pad Software, Inc., La Jolla, CA, USA), and a p=5% or less was considered significant.

RESULTS

In the study of concentration-dependent antioxidative activities, almost all samples were found to be the most effective at 125 µg/mL drug concentration. A drug concentration of 15.25 µg/mL was found to not work for any of the test samples. Unpredictably, in almost all test samples, 1000, 750, and 500 µg/mL drug concentrations were seen to be successively less protective than 125 µg/mL concentrations. Noteworthy, for most cases, 31.5 and 62.5 and 125 and 250 µg/mL concentrations were seen to exhibit non-significant changes in DPPH scavenging potential; hence, values of scavenging activity of test extracts at 31.5, 125, and 500 µg/mL concentrations have been mentioned here.

The study of solvent-dependent antioxidative activities revealed clear-cut differences among the results. At the same drug concentration,

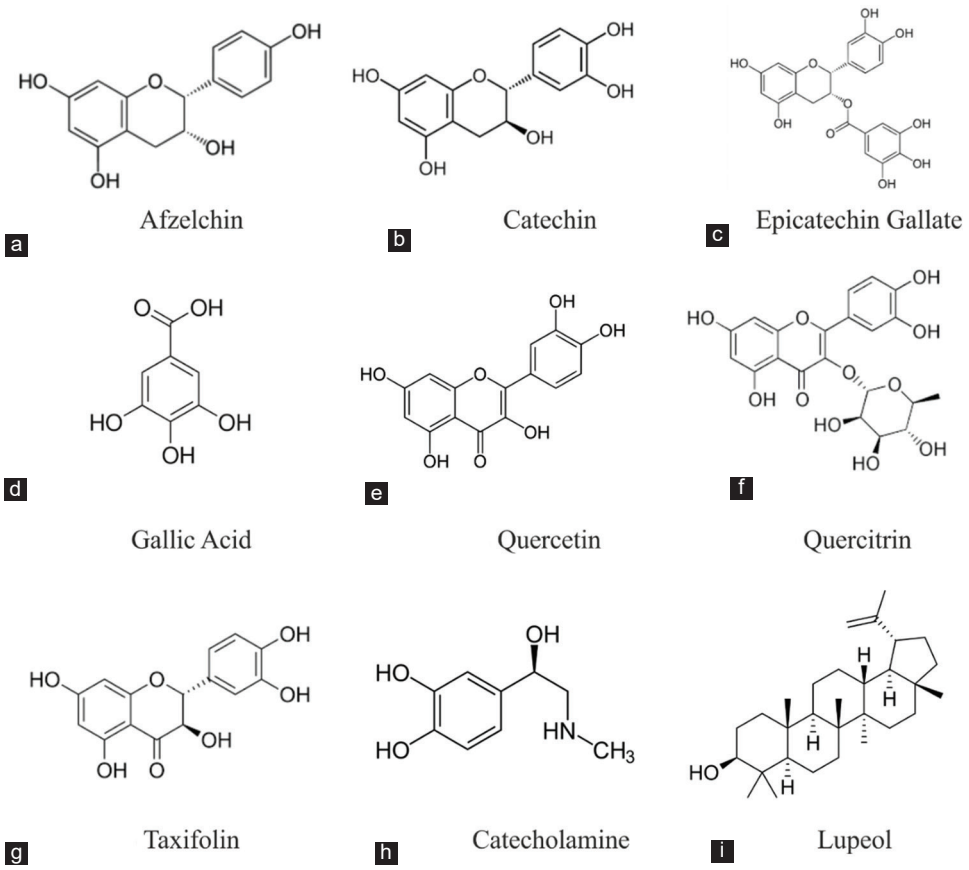

Fig. 1 Chemical structures of (a) afzelechin, (b) catechin, (c) epicatechin gallate, (d) gallic acid, (e) quercetin, (f) quercitrin, (g) taxifolin, (h) catecholamine, (i) lupeol

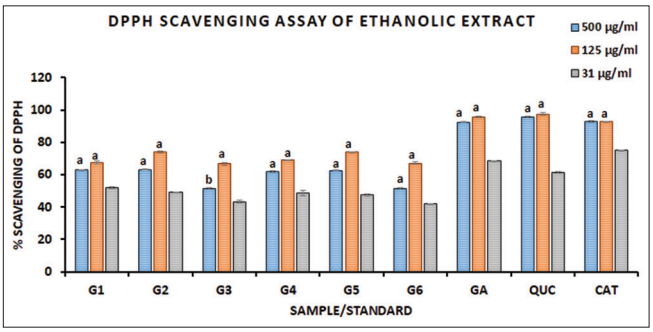

Fig. 2: Comparison of DPPH free radicals scavenging efficacy of methanolic extracts at different concentrations. Data are expressed in % inhibition (Mean±SE of n=5 samples of each group) in comparison to that of the corresponding lowest drug concentration used ([a]p<0.01 and [b]p<0.05). Where G1 (Group-1 samples), G2 (Group-2 samples), G3 (Group-3 samples), G4 (Group-4 samples), G5 (Group-5 samples), G6 (Group-6 samples), GA (gallic acid), QUC (quercetin), and CAT (catechin)

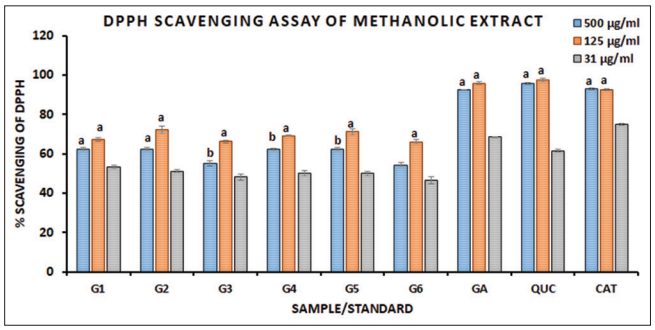

Fig. 3: Comparison of DPPH free radicals scavenging efficacy of ethanolic extracts at different concentrations. Data are expressed in % inhibition (Mean ±SE of n=5 samples of each group) in comparison to that of the corresponding lowest drug concentration used ([a]p<0.01 and [b]p<0.05). Where G1 (Group-1 samples), G2 (Group-2 samples), G3 (Group-3 samples), G4 (Group-4 samples), G5 (Group-5 samples), G6 (Group-6 samples), GA (gallic acid), QUC (quercetin), and CAT (catechin)

methanolic and ethanolic extracts of the same sample group were seen to have equal and the highest DPPH scavenging potential; this was p<0.001 significantly higher than chloroform and benzene extracts at each mentioned (31.5, 125, and 500 µg/mL) equivalent drug concentrations (Figs. 2 and 3). On the other hand, the above-mentioned extracts showed higher (p<0.05 or more) free radical scavenging than acetone extract at corresponding 31.5 and 500 µg/mL concentrations. Though, for most of the concentrations, aqueous extracts at respective concentrations exhibited less scavenging (p>0.05) when compared with methanolic and ethanolic extracts (Fig. 4). However, this exhibited at least p<0.01 significantly greater scavenging than both chloroform and benzene extracts at corresponding concentrations. Both aqueous extract and acetone extract were said to have almost the same DPPH scavenging power at 500 and 125 µg/mL concentrations. However, at 31.5 µg/mL concentrations, some groups' aqueous extracts were seen to have better (p<0.05) scavenging. The scavenging potential of acetone extract is shown in Fig. 5; this exhibited higher (p<0.05 or more) values than both chloroform and benzene extracts. At 500 µg/mL drug concentrations, chloroform extract exhibited higher protection (p<0.05 or more) but not at 125 and 31.5 µg/mL drug concentrations (Fig. 6). In all cases, benzene extracts showed the least free radical scavenging (Fig. 7). The standard antioxidants gallic acid, quercetin, and catechin

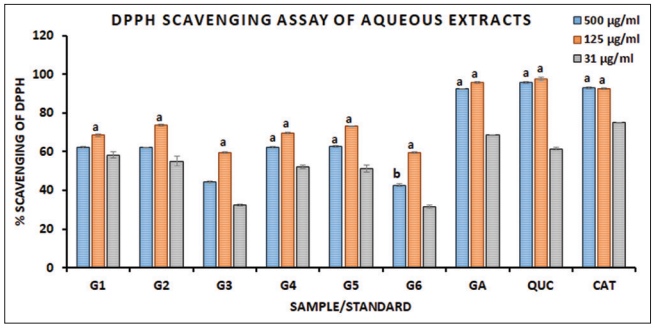

Fig. 4: Comparison of DPPH free radicals scavenging efficacy of aqueous extracts at different concentrations. Data are expressed in % inhibition (Mean±SE of n=5 samples of each group) in comparison to that of the corresponding lowest drug concentration used ([a]p<0.01 and [b]p<0.05). Where G1 (Group-1 samples), G2 (Group-2 samples), G3 (Group-3 samples), G4 (Group-4 samples), G5 (Group-5 samples), G6 (Group-6 samples), GA (gallic acid), QUC (quercetin), and CAT (catechin)

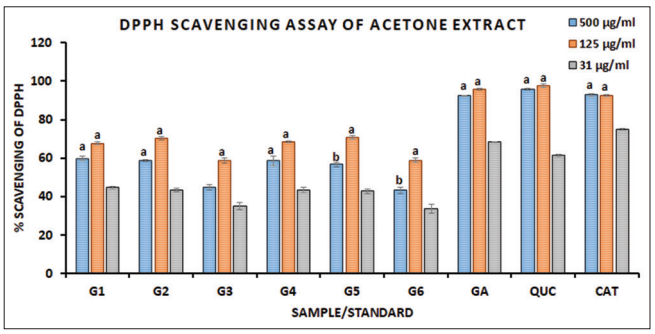

Fig. 5: Comparison of DPPH free radicals scavenging efficacy of acetone extracts at different concentrations. Data are expressed in % inhibition (Mean±SE of n=5 samples of each group) in comparison to that of the corresponding lowest drug concentration used ([a]p<0.01 and [b]p<0.05). Where G1 (Group-1 samples), G2 (Group-2 samples), G3 (Group-3 samples), G4 (Group-4 samples), G5 (Group-5 samples), G6 (Group-6 samples), GA (gallic acid), QUC (quercetin), and CAT (catechin)

were reported to show considerably greater protection (p<0.0001) than all test extracts (Table 2).

The study of seasonal-dependent antioxidative activities also produced worthwhile outputs, as depicted in Table 3. When test samples were collected in the winter and summer, they had higher scavenging rates (p<0.05 or more) for all drug concentrations except for 125 µg/mL drug concentrations in ethanolic and methanolic extracts. However, both winter and summer season samples showed exactly equal protection against the studied parameter. In addition, there was no observed statistically significant difference among the samples of the same seasons collected in 2016 and 2017 across all tested concentrations. In this way, we can say that 125 µg/mL drug concentrations of ethanolic and methanolic extracts collected in the summer or winter can be more effective at getting rid of DPPH.

DISCUSSION

Among all the test extracts, methanolic and ethanolic extracts proved to be more potent antioxidants than all other types of test extracts. As we have already seen, the different solvent-based extracts' ability to get rid of free radicals is because their phytochemistry is different [1,5,34]. The use of organic solvents, such as ethyl acetate, ethanol, methanol, hexane, and chloroform, for extraction purposes results in the production of compounds that possess unique chemical compositions. Consequently, these compounds exhibit various physiological and pharmacological effects [26,32-35].

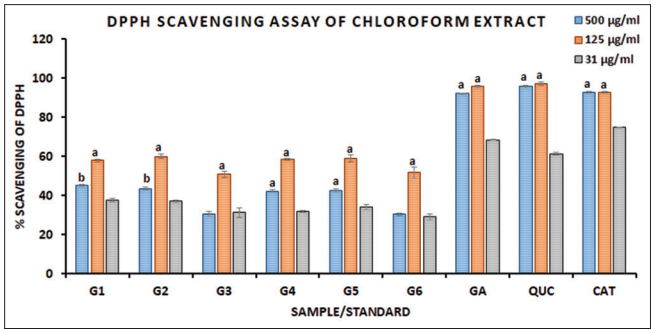

Fig. 6: Comparison of DPPH free radicals scavenging efficacy of chloroform extracts at different concentrations. Data are expressed in % inhibition (Mean±SE of n=5 samples of each group) in comparison to that of the corresponding lowest drug concentration used ([a]p<0.01 and [b]p<0.05). Where G1 (Group-1 samples), G2 (Group-2 samples), G3 (Group-3 samples), G4 (Group-4 samples), G5 (Group-5 samples), G6 (Group-6 samples), GA (gallic acid), QUC (quercetin), and CAT (catechin)

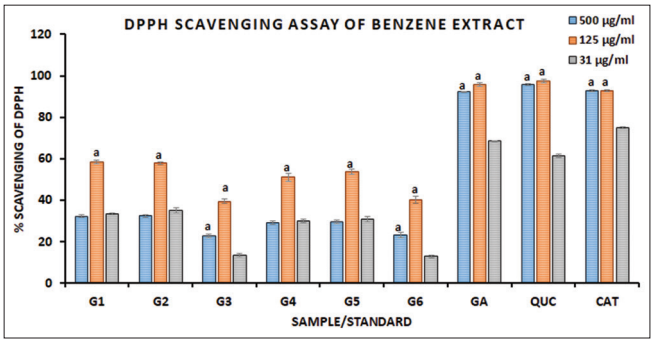

Fig. 7: Comparison of DPPH free radicals scavenging efficacy of benzene extracts at different concentrations. Data are expressed in % inhibition (Mean±SE of n=5 samples of each group) in comparison to that of the corresponding lowest drug concentration used ([a]p<0.01 and [b]p<0.05). Where G1 (Group-1 samples), G2 (Group-2 samples), G3 (Group-3 samples), G4 (Group-4 samples), G5 (Group-5 samples), G6 (Group-6 samples), GA (gallic acid), QUC (quercetin), and CAT (catechin)

The obtained results indicated that at lower concentrations, test plant extracts have sufficient free radical scavenging properties, though higher concentrations of almost all test extracts were seen to be less effective. The lower activity of drugs at higher concentrations may indicate that some of their components act as pro-oxidants or might be taking part in some other metabolic activities that decrease the overall protective activity of drugs [5,32,36]. And possibly the higher drug concentration may have less efficacy due to possible adverse effects, as have been reported earlier [3,23,33-37]. Similar, concentration-dependent scavenging of DPPH radicles was also reported by earlier scientists [5,19,32-36].

According to earlier research from Tamil Nadu, India, a methanolic extract of the stem bark of *A. catechu* can protect against DPPH free radicals and also show great anti-cancer activity against the MCF-7 cell line [35]. In a different study, it was found that the hydroalcoholic extract of *Acacia suma* heartwood could remove DPPH in a concentration-dependent way. This study also confirmed the responsible agent epigallocatechin's anti-oxidative potential *in vitro* assays [37,38]. Epigallocatechin has also been reported in bark extracts of the A. catechu plant and may act as one of the protective agents against free radical damage [39].

The current study also uncovered potential seasonal fluctuations among the plant specimens gathered throughout the winter, summer,

Table 2: Scavenging of DPPH by various types of extracts at three different concentrations (Study of solvent-dependent antioxidative activities)

Scavenging at 500 µg/mL concentration of test samples						
Standard	**Gallic Acid**		**Quercetin**		**Catechin**	
	92.45±0.14^{c}		95.78±0.56^{c}		92.86±0.54^{c}	
Extracts	**Ethanol**	**Methanol**	**Aqueous**	**Acetone**	**Chloroform**	**Benzene**
G1	62.73±0.54^{c}	62.11±0.88^{c}	62.22±0.51^{c}	59.83±1.31^{c}	45.05±0.78^{b}	32.25±0.68
G2	63.28±0.55^{c}	62.28±0.95^{c}	62.07±0.34^{c}	58.71±0.42^{c}	43.62±0.61^{b}	32.42±0.52
G3	51.53±0.59^{c}	54.93±1.34^{c}	44.25±0.81^{c}	44.79±1.28^{c}	30.58±1.30 ^{b}	23.03±0.85
G4	61.76±0.47^{c}	62.20±0.72^{c}	62.27±0.63^{c}	58.65±2.47^{c}	42.35±0.54 ^{c}	29.27±0.82
G5	62.59±0.22^{c}	62.31±0.73^{c}	62.75±0.63^{c}	56.83±1.22^{c}	42.65±0.92^{c}	29.61±0.75
G6	51.45±0.64^{c}	54.31±1.24^{c}	42.64±0.71^{c}	43.39±1.67^{c}	30.39±0.76 ^{b}	23.23±1.42
Scavenging at 125 µg/mL concentration of test samples						
	95.78±0.65^{c}		97.51±0.9^{c}		92.66±0.45^{c}	
G1	67.44±0.86^{c}	67.31±0.96^{c}	68.51±0.57^{c}	67.73±0.86^{c}	58.03±0.83	58.42±0.69
G2	74.07±0.54^{c}	72.22±1.79^{c}	73.68±0.39^{c}	70.31±0.96^{c}	60.02±1.16	57.92±0.61
G3	66.61±0.99^{c}	66.15±0.82^{c}	59.53±0.34^{c}	58.74±1.18^{c}	50.91±1.44^{c}	39.56±0.95
G4	68.91±0.44^{c}	69.08±0.22^{c}	69.54±0.58^{c}	68.41±0.49^{c}	58.51±0.37^{b}	51.21±1.99
G5	73.83±0.41^{c}	71.15±1.42^{c}	73.07±0.33^{c}	70.90±0.84^{c}	59.14±1.84^{a}	53.86±1.21
G6	67.01±0.76^{c}	66.04±1.13^{c}	59.48±0.63^{c}	58.94±1.03^{b}	51.87±2.86^{b}	40.25±1.69
Scavenging at 31.5 µg/mL concentration of test samples						
	68.57±0.13^{c}		61.45±0.68^{c}		74.9±0.34^{c}	
G1	52.07±0.46^{c}	53.21±1.02^{c}	58.24±1.54^{c}	44.70±0.58^{c}	37.76±0.80^{a}	33.39±0.39
G2	49.04±0.27^{c}	51.22±0.61^{c}	54.98±2.55^{c}	43.57±0.83^{c}	37.05±0.74	35.13±1.32
G3	43.34±0.75^{c}	48.16±1.48^{c}	32.58±0.51^{c}	35.19±1.81^{c}	31.31±2.41^{c}	13.51±0.79
G4	48.65±1.45^{c}	50.28±1.35^{c}	52.27±0.88^{c}	43.38±1.41^{c}	32.07±0.43	30.15±0.88
G5	47.41±0.81^{c}	49.99±1.06^{c}	51.38±1.74	42.77±1.01^{c}	34.22±1.28	30.88±1.31
G6	41.73±0.47^{c}	46.58±1.91^{c}	31.75±0.93^{c}	33.57±2.35^{c}	29.11±1.51^{c}	12.91±0.72

Data are expressed as % of scavenging activity (mean±SE of n=5). ^{a}p<0.05; ^{b}p<0.01, and ^{c}p<0.001 were significantly more effective as compared to the respective lowest concentrations, i.e., benzene extract. Where G1 (Group-1 samples), G2 (Group-2 samples), G3 (Group-3 samples), G4 (Group-4 samples), G5 (Group-5 samples), and G6 (Group-6 samples)

Table 3: Scavenging of DPPH by various groups of sample extracts at three different concentrations (Study of seasonal-dependent antioxidative activities)

Scavenging at 500 µg/mL concentration of test samples						
Standard	**Gallic Acid**		**Quercetin**		**Catechin**	
	92.45±0.14^{c}		95.78±0.56^{c}		92.86±0.54^{c}	
Extracts	**Ethanol**	**Methanol**	**Aqueous**	**Acetone**	**Chloroform**	**Benzene**
Winter	62.24±50.36	62.16±51.75	62.25±55.26	59.24±44.04	43.7±34.92	30.76±31.77
Summer	62.93±48.21	62.29±50.6	62.41±53.18	57.77±43.17	43.13±35.63	31.01±33.01
Manson	51.49±42.53	54.62±47.37	43.44±32.17	44.09±34.38	30.48±30.21	23.13±13.21
Scavenging at 125 µg/mL concentration of test samples						
	95.78±0.65^{c}		97.51±0.9^{c}		92.66±0.45^{c}	
Winter	68.18±0.5	68.2±0.8	69.03±0.57	68.07±1.89	58.26±0.66	54.76±0.75
Summer	73.95±0.38	71.69±0.84	73.38±0.48	70.6±0.82	59.57±0.77	55.89±0.63
Manson	66.8±0.62	66.1±1.29	59.51±0.75	58.84±1.47	51.39±1.03	39.9±1.14
Scavenging at 31.5 µg/mL concentration of test samples						
	68.57±0.13^{c}		61.45±0.68^{c}		74.9±0.34^{c}	
Winter	50.36±0.95	51.75±1.18	55.26±1.21	44.04±0.99	34.92±0.61	31.77±0.64
Summer	48.21±0.53	50.6±0.83	53.18±2.15	43.17±0.92	35.63±1.01	33.01±1.3
Manson	42.53±0.61	47.37±1.69	32.17±0.72	34.38±2.07	30.21±1.96	13.21±0.76

Data are expressed as % of scavenging activity (mean±SE of n=5). ^{a}p<0.05; ^{b}p<0.01, and ^{c}p<0.001 were significantly more effective as compared to the respective lowest concentrations, i.e., benzene extract

and rainy seasons. There was no observed statistically significant difference among the samples of the same seasons in both the years 2016 and 2017, across all tested concentrations. In a similar vein, it was shown that the samples collected during the winter and summer seasons had more DPPH-free radical scavenging activity.

The DPPH scavenging test is considered a potential indication of antioxidants since it offers an easy, practical, quick, and replicable method for obtaining results [1,5,34,40]. The assessment of the overall antioxidant capacity of the plant extracts in this study is contingent upon the levels of DPPH free radicals, the concentrations of the extract, and the scavenging efficacy of the bioactive components present in the drug extract [33-35,41]. The use of these antioxidant assays is vital in the investigation of natural sources of antioxidants and their potential applications as functional foods, pharmaceuticals, and food additives [42,43]. Some researchers have measured the antioxidant

activity of the same against DPPH radicals as well as against many other free radicals [33,40-43]. A study also showed that water-based extracts of the plant in question lowered the damage caused by radiation to lipid peroxidation in microsomal preparations of rat liver [5,44]. In this study, 125 µg/mL drug concentrations of ethanolic and methanolic extracts collected in the winter or summer were found to be the most effective at removing DPPH from the environment by plants from the Guna region. Yet, to get more comprehensive and unambiguous knowledge, more research is required, although these findings may provide insights for future investigations in selecting more suitable therapeutic interventions based on concentration, extraction medium, and timeline of sample collection for therapeutic purposes.

Since then, numerous studies have established crucial determinants of oxidative stress in a wide range of illnesses, as well as in the processes of aging and other age-related ailments [35,45]. Oxidative stress arises from a state of imbalance between the levels of antioxidants and oxidants [12,27,34,42]. An important part of figuring out the protective effects of *A. catechu* bark extracts is figuring out how well they fight free radicals. The protective effects of the individual bioactive components, i.e., catechin, quercetin, and gallic acid, also provide firm evidence of their protective potential [35-38,40-43]. Earlier, these natural antioxidants, including other polyphenols and carotenoids, had been documented for a wide range of biological effects, such as anti-inflammatory, antibacterial, antiviral, anti-aging, and anticancer properties [36,42,44-47].

Another study has shown that A. catechu has a notable reservoir of catechins and epicatechins, which are recognized for their potent antioxidant properties [48]. Aqueous extracts from *A. catechu* and *Rotula aquatica* have demonstrated their effectiveness in reducing the oxidative stress that cancer cells cause as well as their potent anti-cancer properties [36]. In the present research, aqueous extracts of bark samples have also been reported for worthful protection at medium-low drug concentrations. Other research has shown that water- and alcohol-based extracts from the bark of *A. catechu* are very good at stopping oxidative stress and cell growth in a number of cancer cell types, such as A549 lung, PC-3 prostate, MCF-7 breast, Hep-G2 liver, HeLa cervix, and IMR32 brain cells [40,49,50]. The potential mechanism of action was discovered to occur through antioxidative pathways [27,34,39]. The study demonstrated that the methanolic and hexane extracts of *A. catechu* bark had greater *in vitro* antiproliferative and cytotoxic effects compared to the aqueous extracts when tested against cancer cell lines [49-51].

Some have proven the anticancer and apoptotic properties of the methanolic extract derived from the heartwood of *A. catechu*. The researchers specifically examined its effects on the human breast adenocarcinoma cell line. The observed extract exhibited significant cytotoxic effects on cultured MCF7 cells and induced apoptosis. We learned from the immunoblot test that the extract caused apoptosis by increasing the Bax/Bcl2 ratio, starting the caspase cascade, and finally cutting polyadenosine ribose polymerase [52,53].

In a different study, the methanolic extract from the heartwood of *A. catechu* exhibited a wide range of biological functions, such as protecting DNA, chelating iron, and acting as an antioxidant. In addition, the extract exhibited antiradical effects against superoxide, nitric oxide, hydrogen peroxide, and hypochlorous acid radicals. The extract that was used in the study included a substantial quantity of phenolic compounds as well as a comparable amount of quercetin [43,54].

The antioxidant properties of *A. catechu* heartwood extract have been shown in several *in vitro* and *in vivo* studies, including in rats, mice, and cell cultures [35-37,40-44]. While the precise mechanisms and molecular pathways may not be fully comprehended in certain instances, several studies have indicated a correlation between the antioxidant properties and the anti-inflammatory, anti-neoplastic, and analgesic effects [45,52-54]. Moreover, some studies have shown the anticancer, analgesic, and anti-inflammatory properties of catechin, which are attributed to the presence of antioxidants in the used extracts [55,56].

Researchers have already found that *A. catechu* heartwood extracts can raise the levels of reduced glutathione (an important natural antioxidant) in cells, make more of a number of antioxidant enzymes work, and lower DNA damage and lipid peroxidation [23,47,53]. The suppression of NF-B activity as well as the regulation of genes linked to inflammation may also have an impact on the effects mentioned in the text. Quercetin, a compound present in the test plant, has been documented for its notable anti-inflammatory, antioxidant, and anti-cancer properties. Catechin and epicatechin are often used as nutritional supplements [44,48,51]. Despite the limited number of *in vivo* and clinical research studies investigating the safety and utilization of *A. catechu* as a dietary supplement, more studies are necessary [32,41,55]. The inhibition of alpha-glucosidase and alpha-amylase activity has demonstrated the anti-diabetic properties of ethanolic bark extracts of *A. catechu* [15-17,43,56,57].

Because of a number of beneficial health impacts and abundant availability in the Guna region, it seemed essential to investigate the possible antioxidant efficacy of the *A. catechu* plant [24,57]. The study of the impact of seasonal changes on free radical scavenging activity was also done, but, to the best of our knowledge, there was no documented information available on the same.

CONCLUSION

Therefore, these findings signify a notable progression in the investigation of the utilization of native plant species for medicinal purposes. This is evidenced by the observed ability of plant extracts to scavenge free radicals, which is influenced by various factors such as the type of solvent used, the concentration of the extract, and the season in which the plant samples were harvested. Moreover, the present investigation has the capacity to provide substantial knowledge about the ideal range of concentration required for the creation of secure commodities. Possibly, this data will be helpful as a basis for examining the further health-promoting and protective potential of the extracts.

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 a Ph.D. candidate.

CONFLICTS OF INTERESTS

None.

SOURCE OF FUNDING

Nil.

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