

Original Article

AN ASSESSMENT OF THE RELATIVE ANTIOXIDANT ACTIVITY OF BARK EXTRACTS OF *ACACIA CATECHU* BY *IN VITRO* FREE RADICAL SCAVENGING ANALYSIS

ARCHANA TIWARI^{1*}, AVINASH TIWARI²

¹Department of Botany, Government P. G. College Guna-473001, (M. P.), India. ²Vice Chancellor, Jiwaji University, Gwalior-474011, (M. P.), India

*Corresponding author: Archana Tiwari; *Email: archanaashish19@gmail.com

Received: 10 Oct 2023, Revised and Accepted: 15 Nov 2023

ABSTRACT

Objective: The objective of this research was to examine and compare the capacity of several bark extracts of *Acacia catechu* to scavenge nitric oxide (NO) free radicals. The study also examined the evaluation of variations in concentration that are reliant on both concentration levels and seasonal changes, using samples obtained throughout various seasons over a span of two consecutive years.

Methods: In this study, six extracts were made utilizing solvents, including ethanol, methanol, aqueous solution, acetone, chloroform, and benzene. In the *in vitro* investigation, a nitric oxide (NO) assay was conducted to evaluate the free radical scavenging efficacy of the test samples.

Results: Out of seven tested sample concentrations, 15.25 µg/ml was reported to be ineffective; higher than 500 µg/ml concentrations (i.e., 705 and 1000) were observed to be less effective than their lower concentrations, while 31.5–500 µg/ml drug concentrations were observed to be protective. Among these three, 125 µg/ml concentrations were found to be most effective ($p < 0.01$ or more). In solvent-based results, methanolic, ethanolic, aqueous, and acetone extracts exhibited at least $p < 0.01$ significant effective NO scavenging, but acetone extract was seen to have comparatively less protection ($p < 0.05$) than the other three extracts. Chloroform and benzene extracts, respectively, showed less protection. Samples collected in the summer season showed greater protection than winter and monsoon.

Conclusion: This study provided a clear observation of the impact of extraction solvent, concentration of drug, and season of sample collection on *in vitro* free radical scavenging potential. These data could help provide possible applications for regional plants for medicinal purposes.

Keywords: Nitric oxide, Catechin, *Acacia catechu* bark extract, Antioxidant, Free radical scavenging, Season-dependent

© 2024 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<https://creativecommons.org/licenses/by/4.0/>) DOI: <https://dx.doi.org/10.22159/ijpps.2024v16i1.49763>. Journal homepage: <https://innovareacademics.in/journals/index.php/ijpps>

INTRODUCTION

The use of herbal treatments produced from plants is a widespread phenomenon throughout the majority of the world. In the majority of developing nations, including India, especially in rural parts, people mostly rely on local traditional medicine, which often includes herbalism, as their primary source of healthcare [1]. Plant-based pharmaceuticals are intricate amalgamations of bioactive substances. The available information about the potential health effects of certain phytochemicals is intertwined with data pertaining to the health outcomes associated with a medication containing these phytochemicals [2]. The potential therapeutic applications of phytochemicals make them promising candidates for medicinal usage [3]. The existence of these bioactive substances in medicinal plants, together with their established pharmacological effects, serves as a scientific basis for the incorporation of these plants into modern medical practices. The plant *Narcissus* has been shown to contain nine distinct alkaloids, among which is the galantamine chemical. This particular compound has demonstrated significant efficacy in the treatment of Alzheimer's disease [3, 4].

Multiple research investigations have provided evidence for the presence of intrinsic antioxidants in many plant species that are used for both culinary and medicinal purposes. Naturally present antioxidants, such as polyphenols, flavonoids, tannins, vitamins, and carotenoids, exhibit a wide array of medicinal attributes, including anti-inflammatory, antibacterial, antiviral, anti-aging, wound healing, and anticancer characteristics, among others [5]. Furthermore, the difference in cost between modern pharmaceutical medications and traditional herbal remedies often makes the latter a more economically viable option with a reduced occurrence of negative consequences [6, 7].

Numerous extant works of literature have documented the historical use of the *Acacia catechu* tree by Ayurvedic practitioners over an extended period of time, specifically for the purpose of managing and

preventing a wide range of health issues [3-5]. Research conducted by the pharmaceutical industry has shown that the phytochemicals included in this particular plant have the capacity to prevent diseases. One illustrative instance is the bioactive component known as catechin, which is found in great quantities inside this particular plant. Catechin exhibits antioxidant qualities and, furthermore, functions as an antibacterial agent. The therapeutic effectiveness of tannin components in wound healing has been acknowledged [5, 7, 8].

There are many bioactive substances in *Acacia catechu* bark, such as catechin, epicatechin, acacatechin, kaempferol, quercetin, quercitrin, tannins, ascorbic acid, riboflavin, thiamine, niacin, gallic acid, and carotenoids, all of which are antioxidants. Scientific research has also shown the antioxidative and free radical-scavenging capabilities of the aforementioned substance [7, 9]. While many individual studies have shown the therapeutic attributes of methanolic, ethanolic, and aqueous bark extracts derived from the *Acacia catechu* plant in various places, there is a scarcity of scientific evidence about these capabilities, specifically in the Guna region [10]. Furthermore, the existing data does not provide an explanation for the relative antioxidative activity of six distinct extracts derived from the same source, which were made using solvents of varying polarity [11].

The primary objective of this research was to examine the comparative antioxidative capacity of various extracts in a concentration-dependent manner with respect to the parameter under investigation. Furthermore, it has been shown that seasonal variations have an impact on the phytochemistry of plants. Therefore, samples obtained during various seasons during two consecutive years were also examined using identical parameters. Given that *in vitro* research provides a simplified model for inquiry, the findings obtained from such studies may serve as indicators of potential impacts in *in vivo* systems. Consequently, preliminary investigations have been carried out using *in vitro* methods as a starting point for the study.

Plant-based drugs are complex mixtures of bioactive compounds. *Acacia catechu* bark extract was observed to be associated with the quality and quantity of secondary metabolites produced by plants. These seem to exert definite physiological actions through either scavenging of disease-causing free radicals or by destruction of infectious agents from the human body. *Acacia catechu* extracts have also played a role in chemistry, with various names of chemicals as catechin, catechol, and catecholamine being derived from bark [6]. *Acacia catechu* is already been reported for the presence of caprylic

acid methyl ester in about higher concentration. Some researches carried out phytochemical studies of *Acacia catechu* and found poriferasterol, poriferasterolacyl glucosides, gallic acid, phlobatannins, d-galactose, aldobiuronic acid, d-rhamnose and l-arabinose etc [7]. *Acacia catechu* bark is known to possess a diverse array of bioactive components, including catechin, epicatechin, acacatechin, kaempferol, quercetin, quercitrin, tannins, ascorbic acid, riboflavin, thiamine, niacin, gallic acid, and carotenoids, which are classified as antioxidants [7-10].

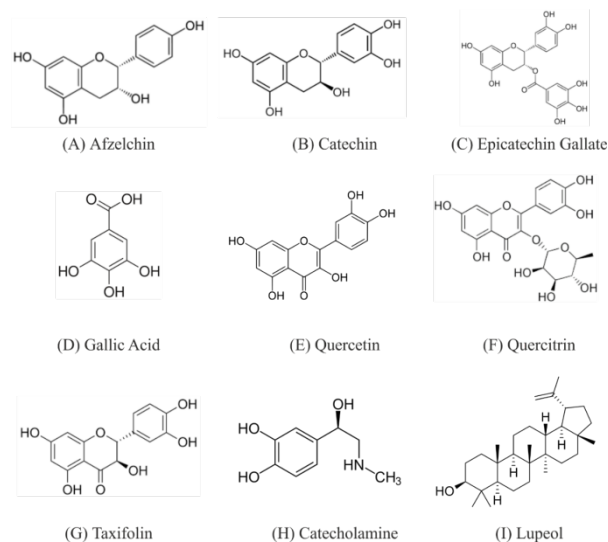


Fig. 1: Chemical structures of (A) Afzelchin, (B) Catechin, (C) Epicatechin gallate, (D) Gallic acid, (E) Quercetin, (F) Quercitrin, (G) Taxifolin, (H) Catecholamine (I) Lupeol

Acacia catechu plants are distributed across the whole nation [10]. This species is often seen in several regions of India, such as Jammu and Kashmir, Punjab, Andhra Pradesh, Himachal Pradesh, Madhya Pradesh, Bihar, Uttar Pradesh, and Orissa, among others. The Lal Khair (red catechu) variety is often seen

in the regions of Deccan, Gujarat, Rajasthan, and southern Maharashtra [7]. This particular variety is widely distributed over many districts of Madhya Pradesh, with a notable presence in the Guna region; this particular species was chosen for our current study project [10-12].

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	<i>Acacia catechu</i> wild-Black cutch

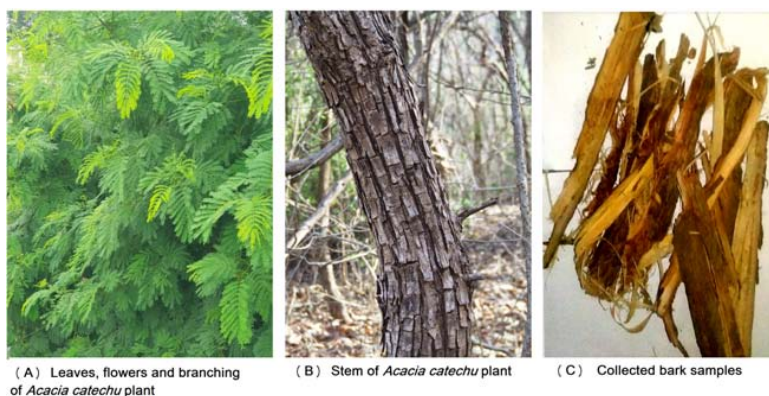


Fig. 2: Morphology and collected bark samples from the *Acacia catechu* plant

MATERIALS AND METHODS

Chemicals

Sodium nitroprusside, gallic acid, quercetin dihydrate, and catechin were purchased from Sigma-Aldrich, St. Louis, MO, USA. While sulphanimide, N-(1-naphthyl) ethylene diamine, H₃PO₄, 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 (SRL) Pvt. Ltd. and from E-Merck (India) Ltd., Mumbai, India.

Collection and processing of bark samples

The bark samples of *Acacia catechu* (specimen deposited in the herbarium of School of studies in Botany, Jiwaji University Gwalior, MP, with voucher number AC-101A-1010/SOB2016 and AC-102A-1020/SOB 2017) were obtained in a random manner from trees located in Biloniya village, Guna (Madhya Pradesh) (Geographical coordinates are L24.650000, A77.320000). The collection area included a circumference of one km. In order to ensure uniformity, the selection of bark was always made at a height of 1.3 meters above the ground. Samples of uniformly round or intact, healthy bark were gathered, subjected to hand cleaning, and then weighed using a portable digital scale. Samples were obtained from a total of five plants during each of the three seasons: winter (in January), summer (in May), and the rainy season (in September). This data collection process spanned two consecutive years, namely 2016 and 2017. In order to differentiate between the seasons and years, the samples collected in 2016 were categorized as groups 1, 2, and 3 for the respective aforementioned seasons, while the samples collected in 2017 were categorized as groups 4, 5, and 6 for the respective aforementioned seasons. In a controlled laboratory setting, materials that had been dried in the shade were pulverized using a mechanical grinder at ambient temperature and then filtered through a fine mesh with a pore size of 0.5 mm. Subsequently, the powdered samples were kept at a temperature of 4 °C [11, 13].

Preparation of various bark extracts

Multiple aliquots of the test specimen were produced using established methods. To get the aqueous extract, a quantity of 50 grammes of powdered bark was subjected to extraction using 1000 milliliters of double-distilled water. The extraction process was carried out at room temperature, using a magnetic stirrer to ensure continuous stirring for a duration of 3 h. Subsequently, the mixture was allowed to stand undisturbed for a period of 24 h, as done earlier [12]. Subsequently, the filtrate underwent a process of desiccation and subsequent determination of its mass. In a similar manner, the organic solvents (namely, 80% ethanol, methanol, benzene, chloroform, and acetone) were prepared by completely combining 50 grammes of dried fine powder obtained from the samples with a volume of 1000 milliliters at ambient temperature. In accordance with the aforementioned protocol, all extracts were made and then subjected to a drying process. The resulting dried extracts were then kept in a refrigerated environment at a temperature of 4 °C. During the experimental period, stock extracts were generated by dissolving dried extracts at a concentration of 1000 µg/ml in distilled deionized water (DDW) for further investigation [11-13].

Nitric oxide (NO) radical scavenging assay

The sodium nitroprusside compound was used as a nitric oxide (NO) radical provider in the free radical scavenging experiment. In order to assess the potential impact of excessive nitric oxide (NO) production on tissues and cells, this experiment was conducted to evaluate the efficacy of the medicine under investigation. In the assay combination, a volume of 0.5 ml of sodium nitroprusside (10 mmol in 0.2 M PBS at pH 7.4) was combined with 0.5 ml of test samples at various concentrations. The mixture was then incubated for a duration of 150 min at a temperature of 20°C while being kept in darkness. Subsequently, a volume of 1 ml of Griess reagent, consisting of 1% sulphanimide and 0.1% N-(1-naphthyl) ethylene diamine in a solution of 2.5% H₃PO₄, was introduced to measure the optical density (OD) at a wavelength of 542 nm relative to a blank sample. The percentage of NO scavenging activity was determined relative to the control. The determination of nitrite concentration was performed by correlating the absorbance values with the amounts of sodium nitrite in standard solutions, as described in other studies [15, 16]. The outcomes were presented as the proportion of nitrite created relative to the control group, which did not include the test sample.

Statistical analysis

The data are presented in the form of the mean ± standard error (SE). The statistical analysis included the use of a one-way analysis of variance (ANOVA), followed by an unpaired student's t-test. The trial version of Prism 9 software for Windows, developed by Graph Pad Software, Inc. in La Jolla, CA, USA, was employed for this purpose. A significance level of 5% or less was deemed statistically significant.

RESULTS

When antioxidative activities that depend on concentration were looked into, it was found that almost all samples worked best when the medication concentration was 125 µg/ml. The medication concentration of 15.25 µg/ml was shown to be ineffective for all of the test samples. Interestingly, it was shown that the medication doses of 1000, 750, and 500 µg/ml consistently exhibited lower levels of protection compared to the 125 µg/ml concentrations across many test samples. It is worth mentioning that, in the majority of instances, concentrations of 31.5 and 62.5 µg/ml, as well as 125 and 250 µg/ml, showed little alterations in their ability to scavenge nitric oxide (NO). Consequently, the values representing the scavenging activity of the test extracts at concentrations of 31.5, 125, and 500 µg/ml have been provided in this context (table 2).

The study of solvent-dependent antioxidative activities revealed clear-cut differences among the results. At the same drug concentration, ethanolic (fig. 3), methanolic (fig. 4), and aqueous (fig. 5) extracts of the same sample group were seen to have significant and equal protection against test parameters; these exhibited $p < 0.001$ significantly greater efficacy than chloroform (fig. 7) and benzene (fig. 8) extracts at 31.5 and 500 µg/ml drug concentrations, while at 125 µg/ml drug concentration the chloroform and benzene extracts were also seen to exhibit significantly high NO scavenging. On the other hand, the acetone extracts have been observed to exhibit moderate NO scavenging potential (fig. 6), as it showed relatively lesser protection than ethanolic, methanolic and aqueous extracts ($p < 0.05$ or more); while greater protection than chloroform and benzene extracts ($p < 0.01$ or more) at almost all tested concentrations.

Table 2: Scavenging of Nitric oxide free radicals by various types of extracts

Scavenging at 500 µg/ml concentration of test samples						
Standard	Gallic acid 60.03±1.08 ^c		Quercetin 62.19±1.31 ^c	Catechin 60.33±1.15 ^c		
Extracts	Methanol	Ethanol	Aqueous	Acetone	Chloroform	Benzene
Group1	32.28±1.17 ^c	37.56±0.32 ^c	36.24±0.75 ^c	35.61±0.78 ^c	25.09±0.57 ^c	19.31±0.41
Group2	36.04±0.51 ^c	37.52±0.73 ^c	37.61±1.03 ^c	34.98±0.66 ^c	28.69±0.41 ^c	18.81±0.63
Group3	30.77±0.58 ^c	27.71±0.83 ^c	29.51±1.08 ^c	26.34±0.75 ^c	19.61±0.84 ^b	13.79±0.51
Group4	31.80±0.45 ^c	34.29±0.79 ^c	34.59±0.82 ^c	32.95±1.39 ^c	24.76±0.58 ^c	17.53±0.49
Group5	38.63±0.82 ^c	37.93±0.13 ^c	38.26±0.38 ^c	35.08±0.75 ^c	25.39±0.55 ^c	17.73±0.45
Group6	32.42±0.62 ^c	29.46±0.74 ^c	24.39±0.77 ^c	26.62±1.03 ^c	22.06±0.69 ^c	13.91±0.85
Scavenging at 125 µg/ml concentration of test samples						
Standard	Gallic Acid 62.19±1.91 ^c		Quercetin 63.32±2.65 ^c	Catechin 60.17±1.32 ^c		

Extracts	Methanol	Ethanol	Aqueous	Acetone	Chloroform	Benzene
Group1	34.75±0.94 ^c	43.23±0.55 ^c	41.92±0.62 ^c	40.32±0.51 ^c	35.97±0.39 ^c	34.58±0.71
Group2	47.95±1.13 ^c	45.51±0.61 ^c	44.06±0.91 ^c	45.36±0.62 ^c	39.67±0.76 ^a	34.28±0.55
Group3	38.42±1.03 ^c	40.05±0.95 ^c	42.51±0.98 ^c	34.55±0.71 ^c	33.51±0.95 ^c	23.29±0.81
Group4	36.78±0.64 ^c	42.62±0.79 ^c	42.06±0.57 ^c	39.87±1.33 ^c	34.84±0.76 ^c	30.61±1.19
Group5	47.47±1.39 ^c	46.15±0.25 ^c	44.83±0.21 ^c	44.32±0.53 ^c	35.22±1.09 ^c	32.25±0.72
Group6	42.06±0.72 ^c	43.51±1.23 ^c	34.18±0.61 ^c	37.52±1.32 ^c	32.63±1.81 ^c	24.12±1.01

Scavenging at 31.5 µg/ml concentration of test samples						
Standard	Gallic Acid		Quercetin	Catechin		
	44.53±1.08 ^c		39.9±5.67 ^c	48.64±2.83 ^c		
Extracts	Methanol	Ethanol	Aqueous	Acetone	Chloroform	Benzene
Group1	27.52±1.08 ^c	29.76±0.26 ^c	31.56±0.45 ^c	25.8±0.84 ^a	20.32±0.45	18.26±0.59
Group2	25.85±1.13 ^a	32.03±1.12 ^c	31.24±1.45 ^c	25.94±0.51 ^a	21.54±0.43	21.04±0.78
Group3	27.68±0.85 ^c	25.35±1.37 ^c	21.67±0.96 ^c	21.44±0.79 ^c	18.86±1.45 ^c	08.09±0.48
Group4	25.39±1.06 ^b	28.79±0.86 ^c	30.44±1.13 ^c	24.37±0.79 ^c	18.61±0.72	17.45±0.85
Group5	32.05±0.68 ^c	26.34±0.44 ^c	28.23±0.96 ^c	26.41±0.62 ^c	20.37±0.76	18.49±0.79
Group6	28.57±1.17 ^c	23.55±0.79 ^c	18.06±0.83 ^c	20.59±1.44 ^c	18.31±0.95 ^c	08.73±0.89

Data are expressed as % of scavenging activity (mean±SE of n = 5). ^ap<0.05; ^bp<0.01 and ^cp<0.001 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), G6 (Group-6 samples).

The investigation of antioxidative activities that vary with the seasons yielded significant results, as seen in table 3. The test samples taken during the summer seasons demonstrated significantly higher scavenging activity (p<0.05 or more) for both

125 and 500 µg/ml drug doses. Furthermore, it was noted that there was no statistically significant disparity among the samples obtained during the same seasons in both 2016 and 2017, regardless of the concentrations that were evaluated.

Table 3: Scavenging of nitric oxide free radicals by various groups of samples

Scavenging at 500 µg/ml concentration of test samples						
Standard	Gallic Acid		Quercetin	Catechin		
	60.03±1.08 ^c		62.19±4.31 ^c	60.3±4.15 ^c		
Extracts	Methanol	Ethanol	Aqueous	Acetone	Chloroform	Benzene
Winter	32.04±0.81 ^c	35.92±0.55 ^c	35.41±0.77 ^c	34.28±1.08 ^c	24.92±0.57 ^a	18.42±0.45
Summer	37.33±0.66 ^c	37.72±0.41 ^c	37.93±0.70 ^c	35.03±0.70 ^c	27.04±0.47 ^b	18.27±0.54
Manson	31.58±0.61 ^c	28.58±0.78 ^c	26.95±0.92 ^c	26.48±0.89 ^c	20.83±0.76 ^b	13.85±0.68

Scavenging at 125 µg/ml concentration of test samples						
Standard	Gallic Acid		Quercetin	Catechin		
	62.19±1.91 ^c		63.32±2.65 ^c	60.17±1.32 ^c		
Extracts	Methanol	Ethanol	Aqueous	Acetone	Chloroform	Benzene
Winter	35.76±0.79 ^c	42.91±0.67 ^c	41.99±0.58 ^c	40.09±0.92 ^b	35.40±0.57	32.59±0.95
Summer	47.71±1.26 ^c	45.82±0.42 ^c	44.44±0.55 ^c	44.84±0.57 ^c	37.43±0.92	33.26±0.63
Manson	40.24±0.87 ^c	41.78±1.09 ^c	38.34±0.79 ^c	36.03±1.01 ^c	33.06±1.37 ^c	23.69±0.91

Scavenging at 31.5 µg/ml concentration of test samples						
Standard	Gallic Acid		Quercetin	Catechin		
	44.53±1.08 ^c		39.9±5.67 ^c	48.64±2.83 ^c		
Extracts	Methanol	Ethanol	Aqueous	Acetone	Chloroform	Benzene
Winter	26.44±1.07 ^b	29.27±0.56 ^c	31.01±0.79 ^c	25.08±0.81 ^c	19.46±0.58	17.85±0.72
Summer	28.95±0.90 ^c	29.18±0.77 ^c	29.73±1.20 ^c	26.17±0.56 ^b	20.95±0.59	19.76±0.78
Manson	28.12±1.01 ^c	24.45±1.08 ^c	19.86±0.89 ^c	21.01±1.11 ^c	18.58±1.21 ^c	08.41±0.68

Data are expressed as % of scavenging activity (mean±SE of n = 5). ^ap<0.05; ^bp<0.01 and ^cp<0.001 significantly more effective as compared to the respective lowest concentrations, i.e., benzene extract.

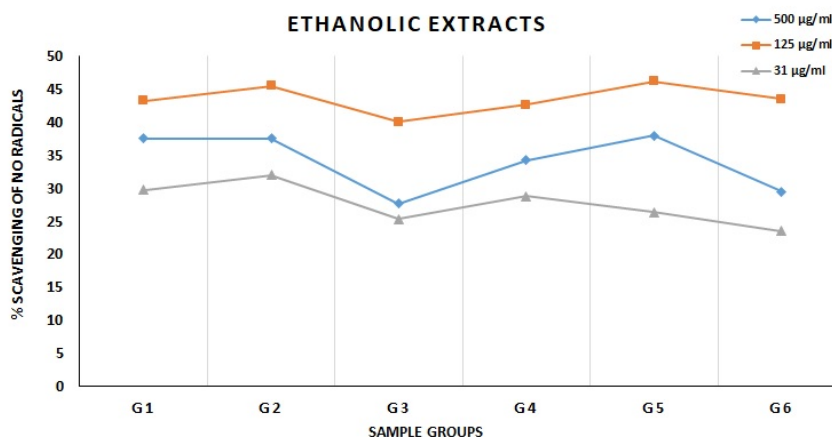


Fig. 3: Comparison of NO free radicals scavenging efficacy of Ethanolic extracts of test samples. Data are expressed in % inhibition (mean±SE of n=5 samples of each group)

Therefore, it can be said that medicine concentrations of ethanolic, methanolic, and aqueous extracts at 125 µg/ml, obtained in the summer, may be more effective at getting rid of nitric oxide (NO). The benzene extracts taken in Manson demonstrated the lowest level of protection against the same.

The graphical representation of the results has been given in fig. 1-6: 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 (standard gallic acid), QUC (standard quercetin), and CAT (catechin).

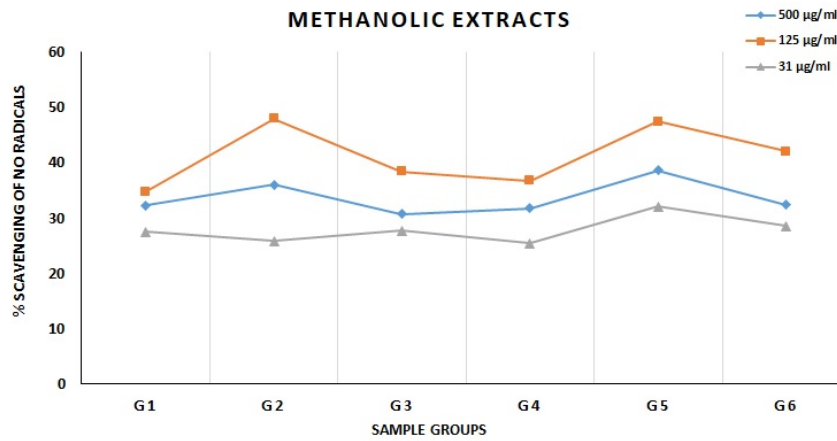


Fig. 4: Comparison of NO free radicals scavenging efficacy methanolic extracts of test samples. Data are expressed in % inhibition (mean±SE of n=5 samples of each group)

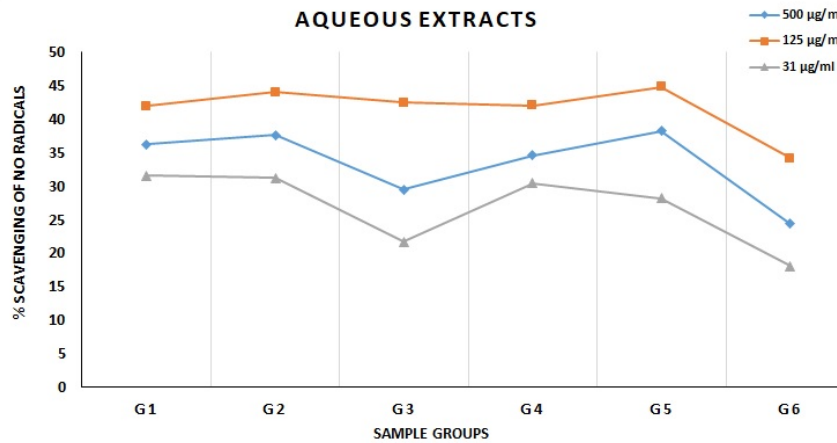


Fig. 5: Comparison of NO free radicals scavenging efficacy aqueous extracts of test samples. Data are expressed in % inhibition (mean±SE of n=5 samples of each group)

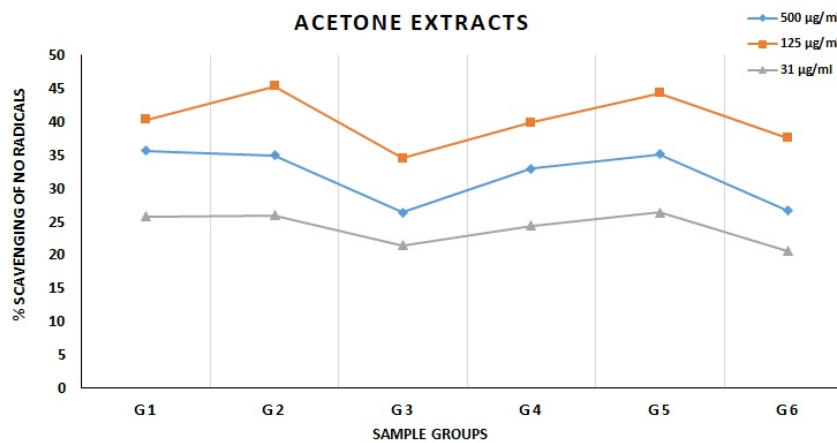


Fig. 6: Comparison of NO free radicals scavenging efficacy acetone extracts of test samples. Data are expressed in % inhibition (mean±SE of n=5 samples of each group)

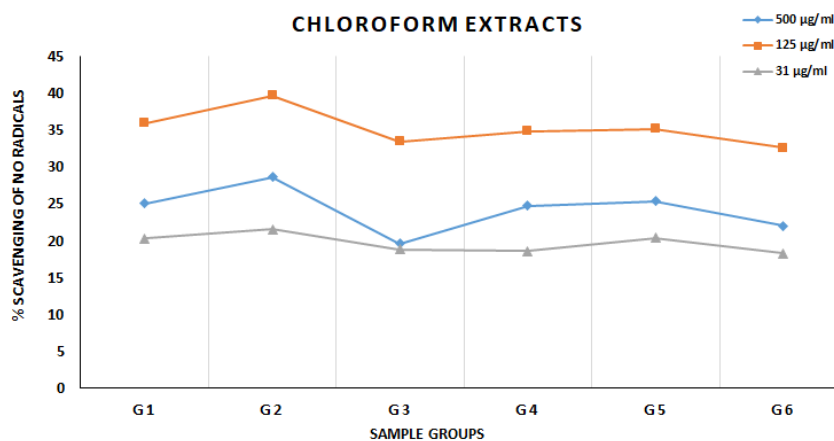


Fig. 7: Comparison of NO free radicals scavenging efficacy chloroform extracts of test samples. Data are expressed in % inhibition (mean±SE of n=5 samples of each group)

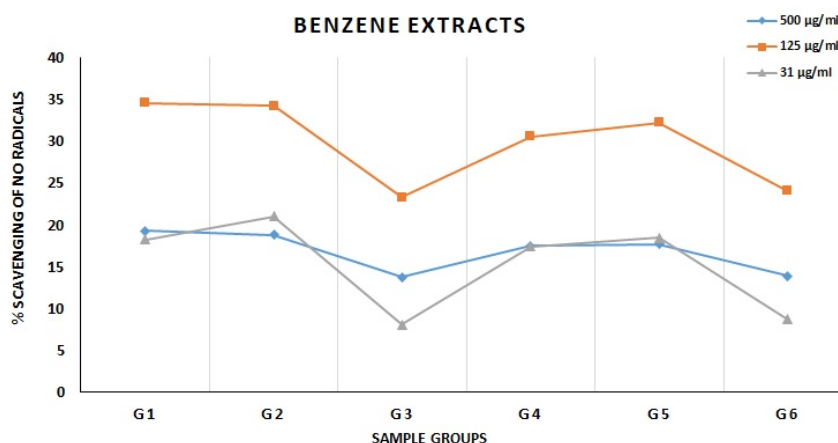


Fig. 8: Comparison of NO free radicals scavenging efficacy benzene extracts of test samples. Data are expressed in % inhibition (mean±SE of n=5 samples of each group)

DISCUSSION

Nitric oxide (NO) is a pivotal cellular signalling molecule within biological systems, playing a critical role in the functioning of immunological, neurological, and physiological systems [3]. While the presence of nitric oxide (NO) is essential for several biological activities, it has been well-documented that an overproduction of NO may result in several pathological conditions, such as oxidative stress, metabolic abnormalities, and impaired wound healing. Iron-containing proteins, such as ribonucleotide reductase, aconitase, guanylate cyclase, and those containing sulfhydryl groups, experience rapid oxidation and subsequent inactivation upon exposure to nitric oxide (NO). The use of NO radical scavengers as therapeutic agents for the purpose of mitigating oxidative damage has significant value [10, 16, 17].

In the current study, it has been shown that all of the test extracts have a significant capacity for scavenging nitric oxide, which is a required feature of medicinal plants. For example, it has been seen that the β -cells of the pancreas are significantly damaged by oxidative stress caused by the NO radical in an *in vivo* system [16]. This damage activates oxidative pathways. Therefore, test medicines that demonstrate a higher efficiency in inhibiting NO may be regarded as highly efficient antioxidants *in vivo* [15]. Additionally, here reduced inhibition at greater doses was also seen, possibly indicating their detrimental effects at higher concentrations [18]. However, throughout the phytochemical screening process, several samples exhibited differences in their phyto-constituents. Moreover, phytochemicals with the ability to scavenge nitric oxide have the

potential to be used as efficacious pharmaceutical agents for mitigating NO-mediated harm [13, 16, 18].

Furthermore, *Acacia catechu* extracts have been shown to control the production of nitric oxide by peritoneal macrophages in a way that depends on the dose given. It has been discovered that the same stimulus elicits the secretion of IL-10, which is a crucial immunoregulator involved in inflammation, while simultaneously inhibiting the synthesis of TNF- α , a mediator of inflammation, that is released by monocytes and macrophages [17-19, 21-23]. In a separate investigation, the researchers examined the efficacy of the 70% methanolic extract derived from the heartwood of *Acacia catechu* in scavenging several radicals, including nitric oxide, peroxynitrite, hydrogen peroxide, singlet oxygen, and hypochlorous acid [20, 24, 25]. Additionally, the various components of the same have already been shown to be effective against a variety of conditions, including skin disorders, melancholia, conjunctivitis, diabetes, haemoptysis, hepato-protective activity, leprosy, body surface infections, leucoderma, colon diseases, helminthiasis, norexia, diarrhoea, dysentery, foul ulcers and wound treatment, haemorrhages, fever, anaemia, and pharyngodynia [16, 18-20]. Owing to its extensive therapeutic properties, it is also often used in a variety of medication compositions. *Acacia catechu*, also referred to as Katha in Hindi, is a dispensed-with component of pan, a betel leaf mixture that is eaten in India and other nations.

The antioxidant activities of *Acacia catechu* heartwood extract have been shown in many *in vitro* and *in vivo* investigations. Although the exact processes and molecular pathways may not be completely

understood in some cases, several studies have shown a link between the antioxidant qualities and the anti-inflammatory, anti-neoplastic, and analgesic benefits [25]. Furthermore, many researches have shown the anticancer, analgesic, and anti-inflammatory effects of catechin, which might be attributed to the existence of antioxidants in the used extracts [17, 21, 22, 26-28]. Given the many advantageous health effects and high prevalence in the Guna area, it was deemed essential to explore the potential antioxidant effectiveness of the *Acacia catechu* plant. The investigation into the influence of seasonal variations on the activity of free radical scavengers was conducted due to the absence of recorded knowledge on this particular subject matter.

CONCLUSION

Consequently, these results represent a significant advancement in the study of the use of native plant species for therapeutic reasons. This is shown by the observed capacity of plant extracts to scavenge free radicals, which is dependent on a number of variables including the kind of solvent used, the extract's concentration, and the time of year the plant samples were collected. Furthermore, this study has the potential to provide significant insights into the optimal concentration range needed to produce safe goods. Perhaps this information might serve as a foundation for additional research into the extracts' ability to preserve and promote health.

ACKNOWLEDGEMENT

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

FUNDING

Nil

AUTHORS CONTRIBUTIONS

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

Declared none

REFERENCES

- Ingle KP, Deshmukh AG, Padole DA, Dudhare MS, Moharil MP, Khelurkar VC. Phytochemicals: extraction methods, identification, and detection of bioactive compounds from plant extracts. *J Pharmacogn Phytochem*. 2017;6(1):32-6.
- Azwanida NN. A review on the extraction methods use in medicinal plants, principle, strength, and limitation. *Med Aromat Plants*. 2015;4(3):1000196.
- Sofowora A. The present status of knowledge of the plants used in traditional medicine in western Africa: a medical approach and a chemical evaluation. *J Ethnopharmacol*. 1980;2(2):109-18. doi: 10.1016/0378-8741(80)90004-5, PMID 7453197.
- Pandey A, Tripathi S. Concept of standardization, extraction, and pre-phytochemical screening strategies for herbal drug. *J Pharmacogn Phytochem*. 2014;2(5):115-9.
- Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga Latha L. Extraction, isolation and characterization of bioactive compounds from plants' extracts. *Afr J Tradit Complement Altern Med*. 2011;8(1):1-10. doi: 10.4314/ajtcam.v8i1.60483, PMID 22238476.
- Das K, Tiwari RK, Shrivastava DK. Techniques for evaluation of medicinal plant products as antimicrobial agents: current methods and future trends. *J Med Plants Res*. 2010;4(2):104-11.
- Reddy MBK, Gowda KPS, Arora AK. Study of wound healing activity of aqueous and alcoholic bark extracts of *Acacia catechu* on rats. *J Pharm Sci*. 2011;1(3):220-5.
- 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. doi: 10.22159/ajpcr.2021.v14i10.43009.
- Jatav BK, Sharma TK, Dassani S. Succession of microfungi on leaf litter of *Acacia catechu* in Datia, Madhya Pradesh, India. *J Pure Appl Microbiol*. 2020;14(1):581-90. doi: 10.22207/JPAM.14.1.60.
- Gajera HP, Patel SV, Golakiya BA. Antioxidant properties of some therapeutically active medicinal plants-an overview. *J Med Aromat Plant Sci*. 2005;27:91-100.
- Rungsung W, Ratha KK, Dutta S, Dixit AK, Hazra J. Secondary metabolites of plants in drugs discovery. *World J Pharm Res*. 2015;4(7):604-13.
- Rimando AM, Olofsdotter M, Dayan FE, Duke SO. Searching for rice allelochemicals: an example of bioassay-guided isolation. *Agron J*. 2001;93(1):16-20. doi: 10.2134/agronj2001.93116x.
- Pal SK, Shukla Y. Herbal medicine: current status and the future. *Asian Pac J Cancer Prev*. 2003;4(4):281-8. PMID 14728584.
- Sahu TR. An ethnobotanical study of Madhya Pradesh 1: plants used against various disorders among tribal women. *Anc Sci Life*. 1982;1(3):178-81. PMID 22556487.
- Tiwari DK, Yadav A. Ethnobotanical investigation of some medicinal plants availed by Gond tribe of Naoradehi Wild Life Sanctuary, Madhya Pradesh. *Anthropologist*. 2003;5(3):201-2. doi: 10.1080/09720073.2003.11890805.
- Agrawal J, Kar A. Chronic treatment of Diabecon in the regulation of alloxan-induced hyperglycemia and oxidative stress in different tissues of adolescent diabetic rats. *Int J Pharm Pharm Sci*. 2014;6(4):83-7.
- Agrawal J, Kar A, Panda S. Synergistic action of phytochemicals augments their antioxidative efficacy: an *in vitro* comparative study. *Asian J Pharm Clin Res*. 2013;6(8):121-6.
- Chaudhari SK, Shalini T, Singh DP, Verma NK, Chandra VA. An overview on *Acacia catechu*. *Int J Res Rev Pharm Appl Sci*. 2012;2(2):342-6.
- Bimla M, Chander J, Kalidhar SB. A review on the chemistry and bioactivity of *Acacia* spp. *J Med Aromatic Plants Sci*. 2005;27(1):51-90.
- Rai R. Promising medicinal plants their parts and formulations prevalent in folk medicines among ethnic communities in Madhya Pradesh, India. *PPIJ*. 2017;5(3):99-106. doi: 10.15406/ppij.2017.05.00122.
- Jain AK, Vairale MG, Singh R. Folklore claims on some medicinal plants used by Bheel tribe of Guna district Madhya Pradesh. *Indian J Trad Knowl*. 2010;9(1):105-7.
- Shah J, Patel S, Bhairy S, Hirlekar R. Formulation optimization, characterization and *in vitro* anti-cancer activity of curcumin loaded nanostructured lipid carriers. *Int J Curr Pharm Sci*. 2022;14(1):31-43. doi: 10.22159/ijcpr.2022v14i1.44110.
- Avalappa H, Padiyappa SD, Pathappa N, Bettadatunga PT, Pramod SN. Legume Indian Bean (*Phaseolus vulgaris*) partially purified anti-nutritional protein factor exhibit anti-oxidant, immunomodulatory and anti-cancer properties. *Int J Pharm Pharm Sci*. 2023;15(2):8-17. doi: 10.22159/ijpps.2023v15i2.46831.
- Sasikala P, Pugazhendy K. Antioxidant and cytotoxic agent from *Acacia catechu* stem bark against MCF-7 cell line. *AJBLs*. 2022;11(1):77-81. doi: 10.5530/ajbls.2022.11.11.
- Baluja S, Chanda S, Solanki A, Kachhadia N. Phytochemical studies of *Acacia catechu*. *Indonesian J Pharm*. 2012;23(4):238-47.
- Negi BS, Dave BP. *In vitro* antimicrobial activity of *Acacia catechu* and its phytochemical analysis. *Indian J Microbiol*. 2010;50(4):369-74. doi: 10.1007/s12088-011-0061-1, PMID 22282602.
- Kumar U, Mishra M, Prakash V. Assessment of antioxidant enzymes and free radical scavenging activity of selected medicinal plants. *Free Radic Antioxid*. 2012;2(3):58-63. doi: 10.5530/ax.2012.3.8.
- Seal T, Pillai B, Chaudhuri K. Effect of cooking methods on total phenolics and antioxidant activity of selected wild edible plants. *Int J Pharm Pharm Sci*. 2023;15(7):20-6. doi: 10.22159/ijpps.2023v15i7.48179.