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RESEARCH ARTICLE

Exploring the Protective Efficacy of Native *Acacia catechu* Bark Extracts Against Fe^{2+} Induced Lipid Peroxidation

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Abstract

Background: *Acacia catechu* is a valuable plant with diverse applications in traditional medicine, industry and ecological management. This plant is abundant in the Guna district of central India but lacks scientific research on its phytochemistry, properties, and medicinal uses. **Aim:** The present study analyzed six different bark extracts (methanol, ethanol, aqueous, acetone, chloroform and benzene) of native *Acacia catechu* for their concentration-dependent, season-dependent (winter, summer, and Manson), and solvent-dependent Lipid Peroxidation (LPO) inhibition potential. **Methods:** In this *in vitro* study, Fe^{2+} ions were used to induce LPO via oxidative stress in liver and kidney tissue homogenate, and the end product of the reaction, i.e., Malondialdehyde (MDA), was measured as MDA formed/hour/mg of protein. The bibliographic analysis was done using the dimention.ai database from 1999 to June 13th, 2024. **Results:** The results of the bibliographic study indicated that earlier no scientific study has been documented on the plant in question. The results of the experimental work indicated a marked increase in MDA level in $FeSO_4$ treated tubes ($p<0.0001$), which was seen to be decreased ($p<0.05$ or less) in bark extracts as well as standard drug-treated tubes ($p<0.0001$). In comparative analysis, the 125 µl/mg concentration was seen to exhibit the highest protection ($p<0.0001$) among all tested concentrations, and the antioxidative capacity was seen in the order of ethanolic>aqueous>acetone>methanolic>chloroform>benzene extracts, though seasonal impacts were inconsistent. Additionally, the pattern of antioxidative activity was seen to be highly correlated ($R^2 = 0.996$) in both liver and kidney tissues. **Conclusion:** The current work may contribute to the comprehension and utilization of the antioxidative characteristics of plant extracts, which might result in notable progress in the fields of medicines, cosmetics, food preservation, agriculture, and environmental management.

Keywords: *Acacia catechu*, Antioxidant Assay, Bibliographic Analysis, Dimentions.ai Database, *In Vitro* Assay, Lipid Peroxidation

Abbreviations: LPO - Lipid Peroxidation; MDA – Malondialdehyde; $FeSO_4$- Ferrous Sulphate; ROS - Reactive Oxygen Species; DNA - Deoxyribonucleic Acid; FRS - Free Radical Scavenging; EGCG - Epigallocatechin Gallate; TBA - Thio-Barbituric Acid; Meth - Methanolic Extract; Eth - Ethanolic Extract; Aqua - Aqueous Extract; Ace - Acetone Extract; Chlo - Chloroform Extract; Benz - Benzene Extract

1. Introduction

For several years, *Ayurveda* has extensively used *A. catechu* (L.f.) Willd. (Table 1) to cure and prevent a range of illnesses[1]. In India, it is locally called the cutch tree, Kahir, cachou, black cutch and Khadira[2]. Recently, many studies have shown the pharmacological characteristics of extracts derived from various components of *A. catechu*, such as heartwood, bark, leaves, seeds, and pods[3]. The heartwood of this plant contains a very effective substance called Katha, which has a broad variety of possible therapeutic uses. The

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chemical analysis of the same revealed the presence of significant quantities of beneficial flavonoids, tannins, and phenolic compounds, including catechin/epicatechin, epigallocatechin, taxifolin, procyanidin, quercetin, and taxifolin[4]. The active compounds, such as catechin or epicatechin, have important roles as anti-inflammatory and antioxidant agents. Similarly, tannins are responsible for the astringent action in the human body[2-4]. The bark extract of this plant has shown many pharmacological effects, such as immunomodulators, anti-hyperglycemic, antinociceptive, antihyperlipidemic, and antiulcer properties[5]. A substantial body of research is demonstrating the considerable antioxidative or free radical-scavenging capabilities of this plant[4,5].

On the other hand, numerous studies have identified Reactive Oxygen Species (ROS) as the primary cause of nearly all types of metabolic disorders. ROS that builds up can oxidize DNA, proteins, enzymes, regulatory molecules, cytokinin, and lipids. This can change their structure, activity, and physical properties, and it could even destroy the cells[6].

Lipid peroxides are increasingly recognized as important factors in several pathological conditions, such as inflammation, diabetes, cancer, neurological diseases, cardiovascular issues, liver damage, and degeneration of the eyes and kidneys[7]. LPO, which is the measurement of lipid peroxides, is a reliable way to check how much ROS is made by living things. LPO tests are useful techniques for studying disorders associated with oxidative stress, evaluating the antioxidant potential of compounds, and examining oxidative stress-related diseases[8]. They offer a deeper understanding of the mechanisms that underlie LPO and assist in the identification of potential therapeutic targets. Moreover, *in vitro* studies on LPO mechanisms and molecular pathways provide a valuable means of comprehensively understanding the process. As a result, researchers worldwide have extensively investigated Free Radical Scavenging (FRS) or anti-LPO properties to gain a more comprehensive understanding of the potential protective effects of the test medication sample(s)[6-8].

Plants belonging to the genus *Acacia* are widely distributed throughout India, especially in the state of Madhya Pradesh. A significant portion of the population in the Indian state of Madhya Pradesh adheres to the traditional system of medicine, which prioritizes the use of herbal remedies as the main approach for both preventing and treating diseases[9]. Furthermore, the relatively exorbitant price of contemporary drugs makes traditional herbal therapies a cost-effective substitute for impoverished individuals in these regions[10]. Additionally, cures derived from these plants often exhibit less detrimental consequences. Therefore, numerous tribes have historically utilized it. For example, the *Bheel* and other indigenous tribal tribes residing in the Guna region of central India often use the *A. catechu* shrub for numerous medicinal reasons[10,11]. Although the scientific evidence for its properties and medicinal efficacy is limited, the literature review and bibliographic outputs also confirm the scarcity of clinical or pre-clinical research on these native *A. catechu* plants[12]. Thus, scientific research on these plants is crucial for exploring their pharmacological potential, discovering novel compounds, ensuring safety and efficacy, and advancing pharmaceutical development[9,11,12].

To fill this research gap, a scientific study was conducted, including an analysis of phytochemicals, antioxidative and various other medicinal properties with different dosages, methods of intake, etc. To determine the seasonal impact on the above-mentioned protective efficacies, plant samples collected in different seasons (i.e., Winter, Summer and Manson) were also comparatively studied[12].

The present piece of research work has been divided into three parts. Part A comprises a review of the literature on existing data that could provide both the basis and significance of this research work. Additionally, the scientific literature on *A. catechu* bark

Table 1. Scientific classification of *A. catechu* ((L.f.)[10]

Kingdom	**Plantae**
Phylum	*Tracheophyta*
Clade	*Angiosperms*
Division	*Magnoliophyta*
Class	*Magnoliopsida*
Subclass	*Rosidae*
Order	*Fabales*
Family	*Fabaceae*
Genus	*Acacia* Mill.
Species	*A. catechu* Willd – Black cutch

extracts for their medicinal efficacy, including FRS and anti-LPO properties, provides a basis for further study[13]. This was made using databases like PubMed, Web of Science, Google Scholar, Scopus, dimention. ai, etc. Whereas, Part B comprises a bibliographic analysis performed using dimensions.ai. Dimensions is part of Digital Science, a technological business with headquarters in London, United Kingdom (also known as Digital Science and Research Solutions Ltd.). The purpose of a dimension database for scientific evaluation is to efficiently query, analyze, and visualize scientific data by handling and organizing large, complicated datasets[12]. So, bibliographic data was gathered and the same was further analyzed for the nature, categories, and patterns of existing research publications over the last 25 years (1999–May 2024) for the plant and parameter under consideration. Part C depicts our experimental analysis, which examines the protective efficacy of the collected samples against chemically induced tissue LPO.

2. Literature Review and Research Gap

With the growing understanding of the potential health advantages of herbal medicine, there is a rising interest in evaluating the worldwide ranking of herbal medications[14]. India has a plethora of knowledge on plant-based remedies for preventive and therapeutic care. *Ayurveda*, *Siddha, Unani*, and other ancient systems of medicine use thousands of plant species for therapeutic reasons. The "*Artharva Veda*," "*Charak*," "*Sushrita*," and other ancient Indian academics helped chronicle "Indian Medicinal Plants." It's commonly known that the period gathered a lot of plant usage data. India has diverse flora and medicinal herbs. An extensive analysis of existing research indicates that while a substantial amount of study has been conducted on ethnomedicinal plants in India, certain remote areas need further investigation to discover novel traditional medicines[15,16].

Acacia catechu grows nationwide. This plant grows in several Madhya Pradesh areas, but Guna district is densely populated with them, so we chose it for our study[9-11]. Catechins, catechols, and catecholamines are key bioactive components of *A. catechu*, hence the name. These substances originate from the extract known as flavanols. This plant's wood, flowers, seeds, shoots, leaves, etc. are rich in medicinal bioactive compounds. The primary bioactive constituents found in *A. catechu* include catechin, kaempferol, quercetin, ascorbic acid, riboflavin, thiamine, niacin, and carotenoids. Other minor components include dihydrokaempferol, taxifolin, afzelchin gum, glycosides, and kaempferol. The same plant also produces lauric acid, methyl ester, 2-ethyl-3-methyl-1-butene, poriferasterol, poriferasterolacyl glucosides, gallic acid, phlorotannins, d-galactose, aldobiuronic acid, d-rhamnose, and l-arabinose[2-4,10].

This plant's bark contains anti-arthritic, antiangiogenic, antimicrobial, anticancer, antiulcer, mitochondrial adhesion inhibition, and protein kinase inhibitory properties. The major catechin Epigallocatechin Gallate (EGCG) strongly suppresses telomerase enzyme function, according to a study[16]. This enzyme helps cancer cells thrive because it maintains chromosomal ends. Epicatechin-3-gallate can be used to treat periodontal and dental cavities. Because tannins are astringent, they may help humans heal wounds[5,11,15]. Catechin, an antioxidant and antibacterial, is abundant in this plant. Quercetin, a polyphenolic flavonol, is prevalent in *A. catechu* bark. The therapeutic properties of this plant make it valuable[17]. The *A. catechu* plant has antifungal, antiviral, spasmolytic, and hypoglycogenic properties, making it effective in treating asthma, bronchitis, cancer, chest discomfort, diarrhoea, mouth sores, sore throats, ulceration, vitiligo, and wound healing[10,11]. Several references in *Ayurveda* show that *A. catechu* has therapeutic properties, including skin condition treatment. Its bark and heartwood make 'khadirarishta', a popular *Ayurvedic* skin tonic. These seem to scavenge disease-causing free radicals or kill pathogenic organisms within the body. 'Khadir churna' stimulates metabolism and calms pitta and kapha dosha, helping to lose weight. Medicines for gingivitis, stomatitis, sore throat, and mouth ulcers, including mouthwashes and gargles, are widely prepared by *A. catechu*[14,18].

The extracts derived from this plant have been shown to enhance the levels of protective immunoglobulins in the bloodstream. Khadir's anti-inflammatory characteristics make it a useful therapy for arthritis, asthma, and inflammatory bowel diseases[1,12]. It may also prevent osteoarthritis cartilage damage by inhibiting pro-inflammatory pathways. *Khadira* powder promotes

new skin cell growth, giving a beautiful, healthy glow. The healthy and regular absorption of nutrients improves gastrointestinal and cellular comfort[2-4,12,14]. Similarly, ethanolic bark extracts of *A. catechu* showed anti-diabetic effects by blocking the activity of α-glucosidase and α-amylase. Experimentations have shown that the treated rats exhibited decreased aspartate, alanine, and alkaline phosphatase activity compared to controls (untreated). In addition, the therapy also resulted in an improvement in blood HDL levels and a drop in triglyceride serum, total cholesterol, VLDL, and LDL. Reports also suggest that the ethanolic extract of *A. catechu* inhibits peptic ulcers[5,14,18,19].

Several *in vitro* studies have examined *A. catechu* extracts for antioxidant activity. Guleria *et al.,* compared the antioxidant properties of *A. catechu* heartwood extract. They observed that the extracts prevented DNA strand breakage. Both methanol and ethyl acetate heartwood extracts possess antioxidant and DNA-protective effects[20]. Heartwood extract fights hydroxyl, superoxide, and DPPH radicals. Studies have shown that the bark extracts of this plant possess substantial antioxidant potential, as they show strong suppression of lipid peroxidation, reducing power, and free radical scavenging properties. These characteristics are primarily due to the extracts' high phenolic content, which effectively stabilizes unstable free radicals by giving them an electron[1,10,21].

Ayurvedic and other traditional medical systems have long used *A. catechu* to treat a variety of illnesses, demonstrating its medicinal potential. These conventional uses are supported by contemporary scientific research, which emphasizes the bark's potential as a natural antioxidant source that may be used to create pharmaceutical formulations and nutraceuticals targeted at treating oxidative stress-related illnesses[3,11]. Previous research has shown that *A. catechu* bark extracts of test plants have anti-lipid peroxidative and free radical scavenging properties. *In vitro,* tests on different types of cancer cells showed that methanol and hexane extracts of the bark were much more effective than water extracts at stopping cell growth and killing cells. These substances are potent antioxidants that imitate the effects of insulin, alleviate diabetes, and improve cardiac function[18,22].

Even though there is sufficient data to back up *A. catechu*'s medical applications around the globe, scientific literature also demonstrated that environmental and geological factors may alter the medicinal behaviour of the same[11]. Environmental factors such as temperature, soil composition, humidity, and sunlight exposure primarily determine the therapeutic qualities of plants. These factors influence the synthesis and accumulation of bioactive compounds within plant tissues, ultimately affecting their efficacy as medicinal agents. For example, temperature can change the rate at which plants break down food and the enzymes that help make secondary metabolites like alkaloids, flavonoids, and phenolic compounds, which are often what give plants their medicinal properties[9,21]. Additionally, soil composition and nutrient availability directly influence plant growth, development, and the production of phytochemicals. Plants grown in nutrient-rich soils often have higher amounts of bioactive chemicals than those grown in nutrient-deficient conditions[23]. Furthermore, environmental stressors such as drought, salinity, and pollution can trigger the production of phytochemicals as a defence mechanism, enhancing the medicinal potential of certain plants[24].

Hence, studying traditionally used plants for their medicinal properties is critical to filling this research gap. Additionally, the research aimed to validate traditional knowledge, discover new drugs, explore biodiversity, and promote cultural understanding of native vegetation[14,22,24]. Possibly, it also helps to reveal a holistic approach to healthcare that integrates scientific research and traditional wisdom, ultimately benefiting both human health and the natural world.

3. Materials and Methods

3.1 Bibliographic Analysis

The Dimensions database was used as the primary source of research publications and further bibliographic analysis[9,11,25,26]. Data on the number of publications, datasets, patents, clinical trials, policy documents, and types of publication (from global to local scenario) was gathered from 1999 up to June 13th, 2024 (1 p.m.) using different keywords such as "lipid peroxidation, *A. catechu*, liver, kidney, *in vitro* study, etc.," in different combinations (mentioned in Tables 1 and 2). To get a broader understanding of the same, the number of publications in different research categories along with the year-wise pattern of publications have also been studied and described for each above-mentioned case.

3.2 Chemicals

While phosphoric acid, toluene, ethyl acetate, n-hexane, ethyl ether, ethanol, benzene, methanol, Thiobarbituric Acid (TBA), Trichloroacetic Acid (TCA), sulfuric acid, sodium bisulphate-monohydrate, Folin and Ciocalteus Phenol (FCP) reagents were supplied by Hi Media Laboratories Ltd., Mumbai, India, gallic acid, quercetin dihydrate, L-ascorbic acid, catechin, and Dimethyl Sulfoxide (DMSO). Sisco Research Laboratories (SRL) Pvt. Ltd. and E-Merck (India) Ltd., Mumbai, India, are the suppliers of malondialdehyde (MDA), Sodium Dodecyl Sulphate (SDS), Ferrous Sulphate ($FeSO_4$), Sodium Chloride (NaCl), Sodium Sulphate anhydrous (Na_2SO_4), Sodium Hydrogen Sulphate ($NaHSO_4$), Sodium Hydroxide (NaOH), Ethylene Diamine Tetra-Acetic Acid (EDTA), sodium potassium tartarate, Hydrogen Peroxide (H_2O_2), Tris (hydroxy-methylamine), Glacial acetic acid, and all other reagents were obtained from Sisco Research Laboratories (SRL) Pvt. Ltd.

3.3 Collection and Processing of Bark Samples

The bark of *A. catechu* trees was randomly selected at DBH (Diameter at Breast Height), which is approximately 1.3 meters above the ground, in Biloniya village, Guna (MP). The specimen was deposited in the herbarium of Jiwaji University Gwalior, MP, with voucher numbers AC-101A/SOB2016 to AC-101O/SOB2016 and AC-101A/SOB2017 to AC-101O/SOB2017. The trees were located within a one-kilometre area. Utilizing a forestry "diameter tape," the average diameter of the seedlings was approximately 19±3 inches. An axe was employed to remove approximately 50 mm of circular or fragmented bark. A digital Vernier calliper (accuracy of 0.01 mm) was employed to measure the thickness of the manually cleaned sample bark on site, and a portable digital balance with an accuracy of 0.001 mg was employed to measure the moist mass. For two consecutive years, 2016 and 2017, samples were randomly collected from five different plants during each season: winter (in January), summer (in May), and monsoon season (in September). The bark samples were subsequently dried for four weeks, then ground using a mechanical grinder at room temperature and filtered through a fine sieve (0.5 mm). Additional studies were performed on these finely ground materials, which were then stored at a temperature of 4°C[9-11,21].

3.4 Preparation of Various Bark Extracts

Different test sample extracts were made with a technique previously used[11,21]. To prepare the aqueous extract, 50 grammes of bark powder were extracted using 1000 millilitres of double-distilled water, or at a ratio of 1/20 w/v, at room temperature (RT) while being continuously stirred for three hours using a magnetic stirrer, and then left for twenty-four hours. We then dried and weighed the filtrate. Similar to this, 50 gm of dried fine powder samples were thoroughly mixed with 1000 ml volume (i.e., at a ratio of 1/20 w/v) of the appropriate solvent at room temperature to prepare extracts of organic solvent (i.e., 80% ethanol, methanol, benzene, chloroform, and acetone). These were placed on a shaker set at 150 rpm for 12 hours, and then they were left motionless for the next 24. The solutions were filtered with a muslin cloth filter and refiltered with a Whatman filter No. 1. To get a pure extract, these filtrates were further concentrated by fully evaporating the corresponding solvents under low pressure. For further processing, the dehydrated extracts were stored at 4° C. At the time of the experiment, dry powders were dissolved in brand-new double-distilled water of the required concentration[9-11,21].

3.5 Animals

The research used genetically uniform Swiss albino mice that were selectively bred to achieve genetic uniformity. The mice were aged between 2 and 2.5 months and had an average weight of around 28±2 g. The subjects were housed in polypropylene enclosures with regulated environmental parameters, which included a consistent temperature of 27±2 °C, humidity levels maintained at 50±5%, and a light-dark cycle of 14 hours of illumination followed by 10 h of darkness. The rats were provided with unrestricted availability of commercial rat meal (Golden Feeds, New Delhi, India) and were given sterilized drinking water. The ethical standards set by the "Committee for Control and Supervision of Experiments on Animals" (CPCSEA), a department under the "Ministry of Social Justice and Empowerment, Government of India," were followed. The registration number of the institutional ethics committee is 1546/PO/E/S/11/

CPCSEA. The experimental animals were handled and cared for in the animal house of ADINA, Institute of Pharmaceutical Sciences, Sagar, Madhya Pradesh, India[10,28,29].

3.6 Preparation of Tissue Homogenate

Since the liver and kidneys have a direct role in drug metabolism, these two organs were taken for LPO estimation. Immediately after the animals were sacrificed, the liver and kidneys were taken. After being cleaned properly with cooled phosphate buffer saline (PBS; molarity 0.1M and pH of 7.4), the organs were placed on an ice tray and sliced. Using a motor-driven Teflon homogenizer (Remi motor, RQ-127 A), sliced tissues were homogenized with PBS to produce a 10% w/v homogenate. This was then centrifuged at 15,000 revolutions per minute for half an hour at 4 ˚C to extract the big debris and get a clean supernatant. This freshly prepared supernatant was further used for an *in vitro* LPO assay[28,29].

3.7 Lipid Peroxidation (LPO) Assay

The LPO test is a commonly used method for measuring antioxidant activity. Malondialdehyde (MDA) is one the major peroxidation products formed during membrane LPO, and when this chemical is heated at a temperature of 100 °C with thiobarbituric acid (TBA) under acidic circumstances, it forms a pink-coloured complex. LPO was induced in liver and kidney tissue homogenate by adding $FeSO_4$ (10 mM in distilled water)[28]. The possible protective effects of the test samples were assessed using five different concentrations (31.25, 62.5, 125, 250, and 500 µg/ml), each in triplicate. The colour intensity was measured at a wavelength of 532 nm using a UV-VIS spectrophotometer, following the methods described by Ohkawa *et al.*, (1979)[30]. In the control tube, distilled water was added in place of $FeSO_4$. The LPO was measured and expressed as the rate of MDA production per hour per milligram of protein. A calibration curve was created by using several concentrations of MDA (ranging from 1 to 10 nm/ml) dissolved in 0.1 M PBS[28-30] (Figure 1).

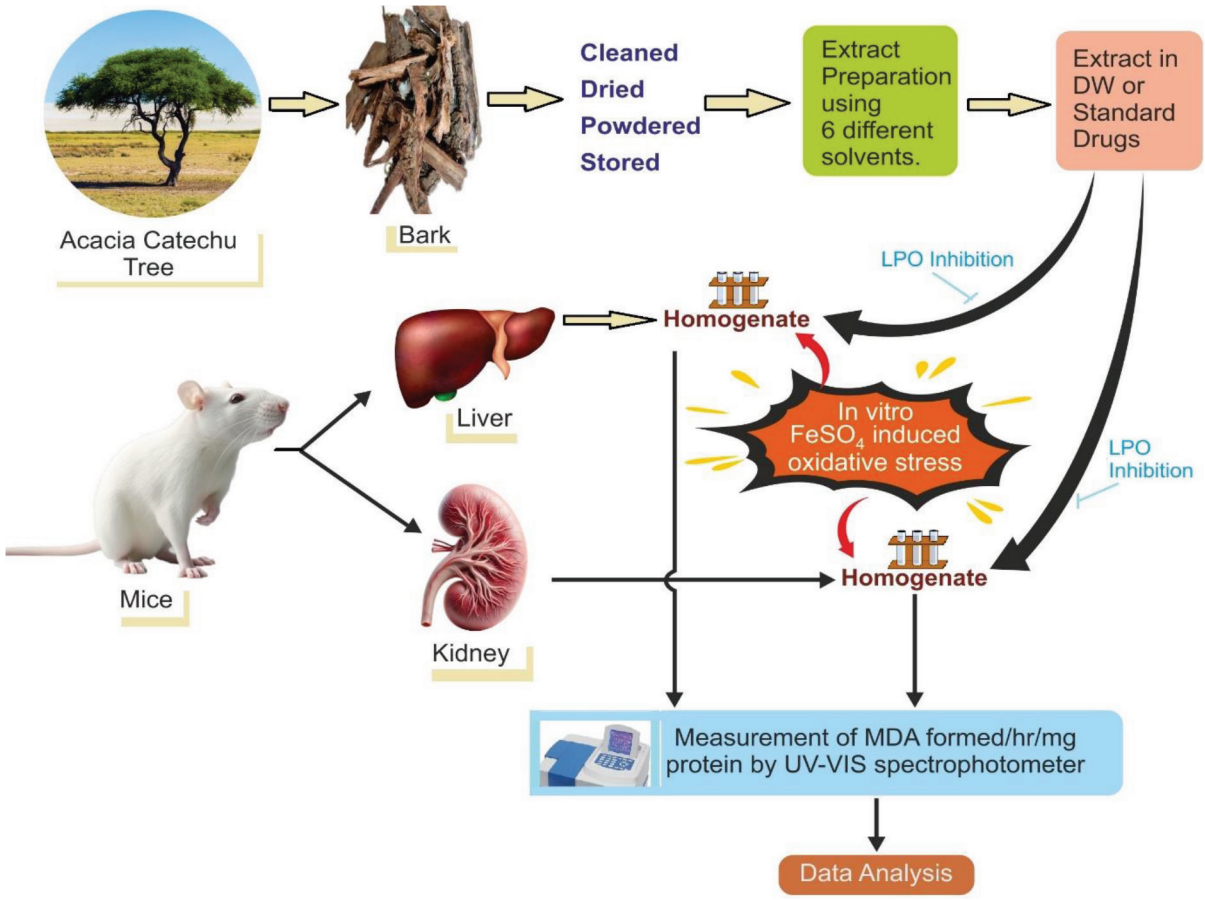


Figure 1. Schematic diagram of the experimental setup.

3.8 Estimation of Tissue Protein

The tissue protein was quantified using the protocol of Lowery *et al.*,[29]. For the assay, 1 ml of tissue homogenate was added to 5 ml of the alkaline copper reagent, which was {2 sodium carbonate in 0.1 M sodium hydroxide (A) + 0.5% copper sulphate in 1% sodium potassium tartrate (B): (A+B) = 100 ml+2 ml}. After vertexing the solution and letting it stand at room temperature for ten minutes, 0.5 ml of the Folin-Ciocalteau reagent—a diluted 1:1 version of commercially available 2N—was added. It was then quickly mixed and left to incubate for an additional thirty minutes at room temperature. The quantity of protein was expressed in milligrams per millilitre (mg/ml) by measuring the absorbance at 660 nm against a blank that contained distilled water instead of the sample[28-30].

3.9 Statistical Analysis

The collected data were analyzed using three criteria, and the outputs were split into three parts to provide a clear picture of the anti-LPO potential of the test samples[21]. A comparative analysis of the polarity and composition of solvents employed to extract the total LPO inhibitory potential was the main focus of Section 1. The purpose of the data analysis in Section 2 was to examine the effects of the winter, summer, and monsoon seasons on the anti-LPO potential of the same set of samples. In Section 3, the samples of both liver and kidney tissues were compared solvent-wise, along with the correlation analysis of LPO inhibition between the same tissues. The values exhibit a mean ± standard error (SE). The statistical studies included one-way analysis of variance (ANOVA), unpaired Student's t-tests, and Pearson's correlation analysis. Statistical significance was defined as a threshold of 5% or below[10,29].

3.10 Data Presentation

In all the experimental research data shown in the figures, the abbreviations and symbols used are as follows: Meth (methanolic extract), Eth (ethanolic extract), Aqua (aqueous extract), Ace (acetone extract), Chlo (chloroform extract), Benz (benzene extract).

4. Results and Discussions

A search using the term "lipid peroxidation" yielded 808,517 publications in the Dimention.ai database. Furthermore, there were 110,134 patents, 3,794 grants, 1,691 datasets, 1,085 policy papers, and 741 clinical trials completed for the same keywords. The 681,376 research articles and 98,453 book chapters, along with 13,575 edited books, demonstrated the importance of this metric in several additional studies and research. Table 2 presents the results of LPO with various keyword combinations, clearly indicating the quantity and variety of grants, publications, and clinical research in a specific field.

These results also showed that out of 808,517 studies, 538,307 have been done around the world in *in vitro* systems, and almost 78,979 have been done in India. Madhya Pradesh had 5,082 similar studies, while Guna district only had 98. The database search using the keywords "*in vitro* study of peroxidation of *A. catechu* extracts in Guna district, Madhya Pradesh, India" showed a total of 3 research articles. These results demonstrated the necessity for comprehensive study in this field in the first place. Secondly, as no clinical trials or pre-clinical studies have been documented in Madhya Pradesh as a whole, the results further underscored the urgent need for more *in vivo* investigations in this area (Table 3).

The year-by-year distribution of publications for each set of keywords in the bibliographic data (Figures 2-4) demonstrated a steady rise in publications in subsequent years, indicating the significance of the parameter under investigation. Figure 4 depicts the paucity of the reports on the plant and parameter under concern. Also, the analysis of research categories for the main keywords again showed a comparatively limited number of scientific studies in fields related to the health, medical, or pharmaceutical sectors (Figures 5 and 6).

The findings of LPO assays showed that the addition of $FeSO_4$ to liver and renal tissue homogenate resulted in a significant increase in LPO by 732.35% ($p<0.0001$) and 483% ($p<0.0001$), respectively. In each of the described tissues, the treatment with the test sample resulted in a significant reduction in tissue LPO compared to the $FeSO_4$-treated group ($p<0.05$ or less).

The concentration-dependent analysis revealed a significant decrease in LPO ($p<0.05$) for each drug concentration from 31.5 to 500 µg/ml, but for both of the studied tissue systems, the highest level of protection ($p<0.0001$) was observed at a drug concentration of

Table 2. The bibliography of a documented database of the number of publications, datasets, grants, patents, clinical trials, and policy documents related to the below-mentioned keywords, retrieved from the dimention

S. No.	Keywords	Publications	Datasets	Grants	Patents	Clinical trials	Policy documents
1	"Lipid peroxidation"	808,517	1,691	3,794	110,134	741	1,085
2	"*In vitro* study of lipid peroxidation"	538,307	132	574	40,890	81	932
3	"*In vitro* study of lipid peroxidation in liver tissue"	296,237	11	51	23,338	17	848
4	"*In vitro* study of lipid peroxidation in kidney tissue"	209,036	5	22	16,974	12	823
5	"*In vitro* study of lipid peroxidation in liver and kidney tissue"	182,828	2	14	15,014	7	814
6	"*In vitro* study of lipid peroxidation in India"	124,391	4	1	2,271	2	506
7	"*In vitro* study of lipid peroxidation in Madhya Pradesh India"	5,082	0	0	12	0	10
8	"*In vitro* study of lipid peroxidation in Guna district, Madhya Pradesh, India"	98	0	0	0	0	1
9	"*In vitro* study of lipid peroxidation in liver tissue in Guna district Madhya Pradesh India"	90	0	0	0	0	1
10	"*In vitro* study of lipid peroxidation in kidney tissue in Guna district Madhya Pradesh India"	84	0	0	0	0	1
11	"*In vitro* study of lipid peroxidation in liver and kidney tissue in Guna district Madhya Pradesh India"	84	0	0	0	0	1
12	"*In vitro* study of peroxidation of *A. catechu* extracts in Guna district Madhya Pradesh India"	36	0	0	0	0	0
13	"*In vitro* study of peroxidation in liver tissue and *A. catechu* bark extracts in Guna district Madhya Pradesh India"	33	0	0	0	0	0
14	"*In vitro* study of peroxidation in kidney tissues and *A. catechu* bark extracts in Guna district Madhya Pradesh India"	33	0	0	0	0	0

Source: https://app.dimensions.ai; © 2024 Digital Science and Research Solutions Inc., Exported: June 13, 2024

125 µg/ml, regardless of the extraction medium used (Figures 7 and 8). Therefore, the results of the 125 µg/ml drug concentration were compared for further seasonal and solvent-dependent LPO inhibitory analysis.

Comparing how well the same drugs work against LPO in different seasons showed that they worked differently in different types of solvents. One study looked at liver tissue homogenate extracts made with ethanol, water, and acetone. They also looked at how the seasons affected the drug's inhibitory activity (at a dose of 125 µg/ml). They found that there was no significant difference ($p>0.05$) between samples taken in the winter, summer, and Manson seasons. Methanolic extracts obtained during the winter and summer seasons had significantly higher protection ($p<0.05$) than those from the Manson season. In contrast, the chloroform and benzene extracts from samples collected during the winter season showed a significantly higher level of LPO inhibition compared to samples from the other two seasons (Figure 9a). With regards to the renal tissue system, all extraction mediums, except for ethanolic ($p > 0.05$) and acetone ($p>0.05$) extracts, showed significant differences ($p < 0.05$) in the same (Figure 9b).

Furthermore, the study of solvents for LPO inhibition revealed significant differences among the extracts used. For example, in liver tissue, ethanolic and aqueous extracts were seen to be more protective than others, while in the renal tissue system, ethanolic, aqueous, and acetone extracts were observed to be comparatively more protective than methanolic, chloroform, and benzene extracts (Figure 10). Overall, the anti-LPO capability of the tissue systems was found to follow the order: ethanolic>aqueous>acetone>meth

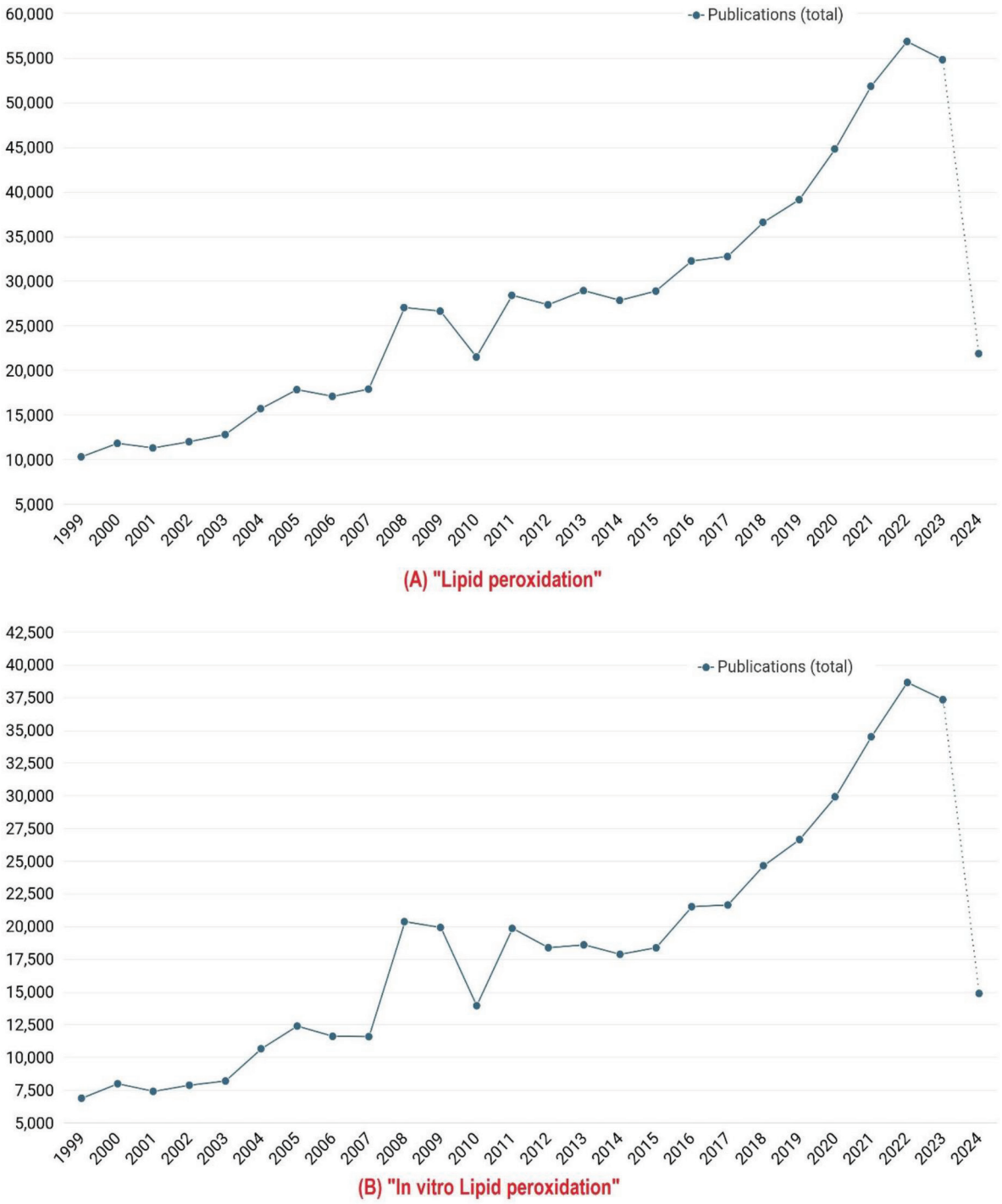
Table 3. The bibliography of an available database of the number of research articles, book chapters, edited books, preprints, monographs and proceeding along with the total number of publications related to the below-mentioned keywords

S. No.	Keywords	Type of publication						Total publications
		Article	Chapter	Edited book	Preprint	Mono graph	Pro ceeding	
1	"Lipid peroxidation"	681,376	98,453	13,575	10,939	2,348	1,826	808,517
2	"*In vitro* study of lipid peroxidation"	434,558	81,237	13,069	6,652	2,078	713	538,307
3	"*In vitro* study of lipid peroxidation in liver tissue"	216,998	62,426	11,641	3,123	1,834	212	296,234
4	"*In vitro* study of lipid peroxidation in kidney tissue"	139,979	54,131	11,027	2,081	1,702	116	209,036
5	"*In vitro* study of lipid peroxidation in liver and kidney tissue"	116,730	52,092	10,582	1,676	1,655	93	182,828
6	"*In vitro* study of lipid peroxidation in India"	78,979	33,856	9,103	1,033	1,341	79	124,391
7	"*In vitro* study of lipid peroxidation in Madhya Pradesh India"	2,803	1,154	1021	82	19	3	5,082
8	"*In vitro* study of lipid peroxidation in Guna district, Madhya Pradesh, India"	15	8	66	0	9	0	98
9	"*In vitro* study of lipid peroxidation in liver tissue in Guna district Madhya Pradesh India"	14	7	60	0	9	0	90
10	"*In vitro* study of lipid peroxidation in kidney tissue in Guna district Madhya Pradesh India"	11	6	58	0	9	0	84
11	"*In vitro* study of lipid peroxidation in liver and kidney tissue in Guna district Madhya Pradesh India"	11	6	58	0	9	0	84
12	"*In vitro* study of peroxidation of *A. catechu* extracts in Guna district Madhya Pradesh India"	3	1	27	0	5	0	36
13	"*In vitro* study of peroxidation in liver tissue and *A. catechu* bark extracts in Guna district Madhya Pradesh India"	3	1	25	0	4	0	33
14	"*In vitro* study of peroxidation in kidney tissues and *A. catechu* bark extracts in Guna district Madhya Pradesh India"	3	1	25	0	4	0	33

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anolic>chloroform>benzene extracts. Further studies also revealed the comparatively equal protective action of ethanolic, aqueous, and acetone extracts as compared to the standard drugs catechin, quercetin, and gallic acid used with respect to their relative concentrations (Table 4). In addition to this, the Pearson correlation analysis shows a strong and statistically significant connection ($R^2 = 0.9919$; $p<0.001$) between the effectiveness of the test samples in reducing lipid peroxidation in the liver and kidney tissues. This correlation holds for all the extraction media that were examined (Figure 10).

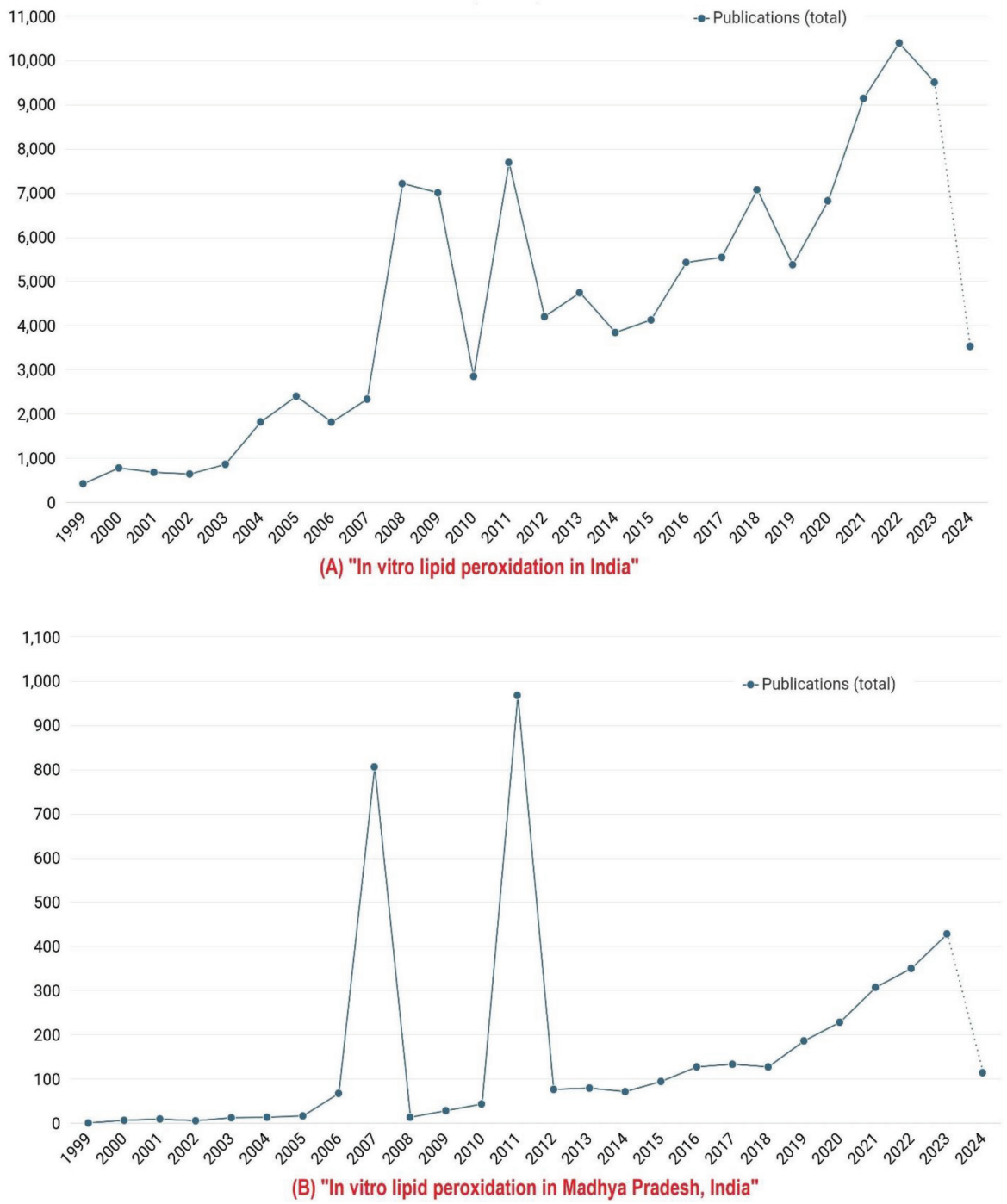
The present investigation demonstrated that the experimental samples have notable antioxidant efficacy in countering oxidative stress in *in vitro* tissue systems. Previous reports have also shown that these extracts can fight free radicals and/or act as antioxidants against the NO free radical test and the DPPH assay[21,31]. Prior to commencing the experimental work, a comprehensive review of the literature and a thorough bibliographic analysis yielded valuable insights[1,7]. Here, the documented literature and results of bibliographic analysis provide valuable ground for the present investigation.



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Figure 2. The bibliography of the total number of publications per year from 1999- June 13th, 2024, using the keywords (A) "Lipid peroxidation", (B) "*In vitro* lipid peroxidation".

Bibliographic analysis is a valuable tool in academic and research settings, offering numerous uses and benefits[10,26]. This is a powerful method for gaining insights into the structure, dynamics, and evolution of research fields. Trend analysis in a bibliographic study involves examining patterns and shifts in research topics over time by analyzing publication and citation data. This method helps identify emerging areas of interest, the evolution of specific fields, and changes in research focus[27,32]. By tracking the frequency of



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Figure 3. The bibliography of the total number of publications per year from 1999- June 13th, 2024, using the keywords (A) "*In vitro* lipid peroxidation in India" (B) "*In vitro* lipid peroxidation in Madhya Pradesh, India".

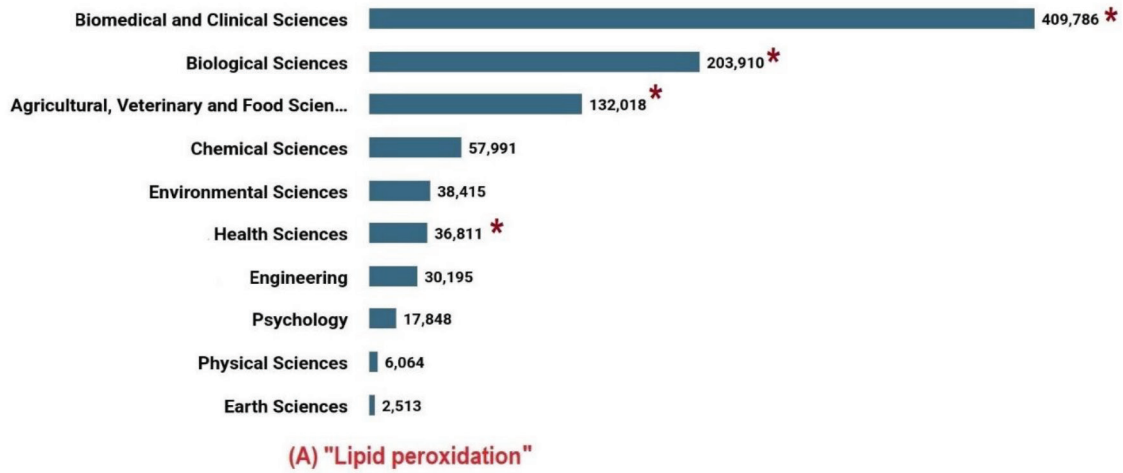
keywords, authorship patterns, and publication venues, researchers can discern which topics are gaining traction and which are declining. This analysis is crucial for understanding the direction of scientific inquiry, anticipating future developments, and guiding strategic decisions in research funding and policy-making[33]. The results of this analysis indicate that the measurement of LPO is a significant parameter for the measurement of the *in vitro* antioxidative efficacy of test drugs. The higher number of publications and

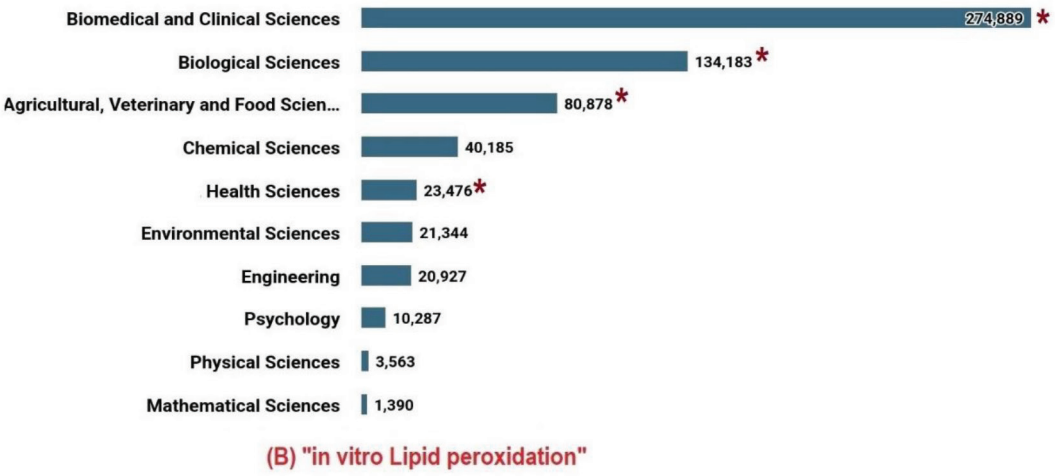


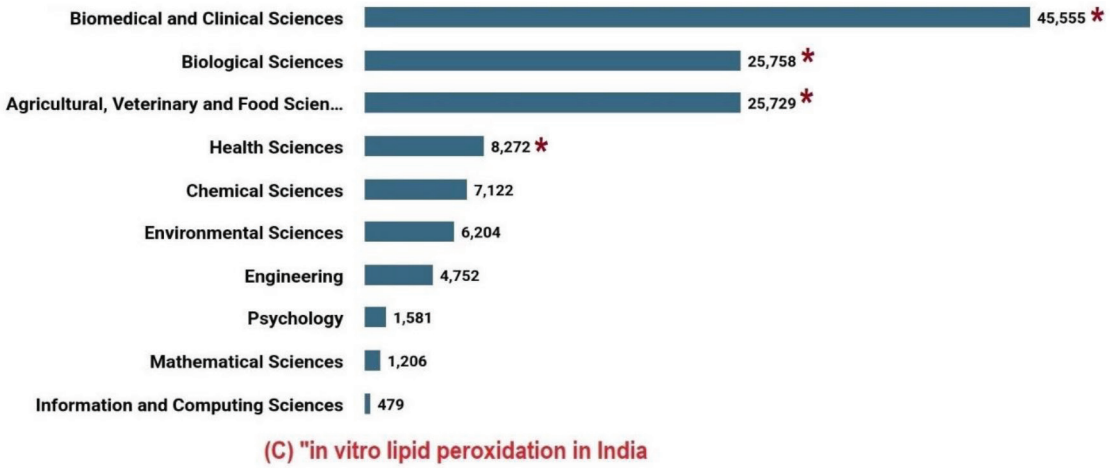
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Figure 4. The bibliography of the total number of publications per year from 1999- June 13th, 2024, using the keywords (A) "*In vitro* lipid peroxidation in Guna District, Madhya Pradesh, India" and (B) "*In vitro* lipid peroxidation in *A. catechu*, Guna District, Madhya Pradesh, India".

the increasing number of publications year by year provide validation for the presented outcomes. This method also helps detect shifts in research focus, the emergence of new subfields, and the overall growth of knowledge in a particular area. Such chronological analysis is invaluable for constructing a detailed and dynamic picture of the research landscape, informing future studies, and shaping academic and funding priorities[31-33]. A research category-wise database in a bibliography involves organizing and analyzing

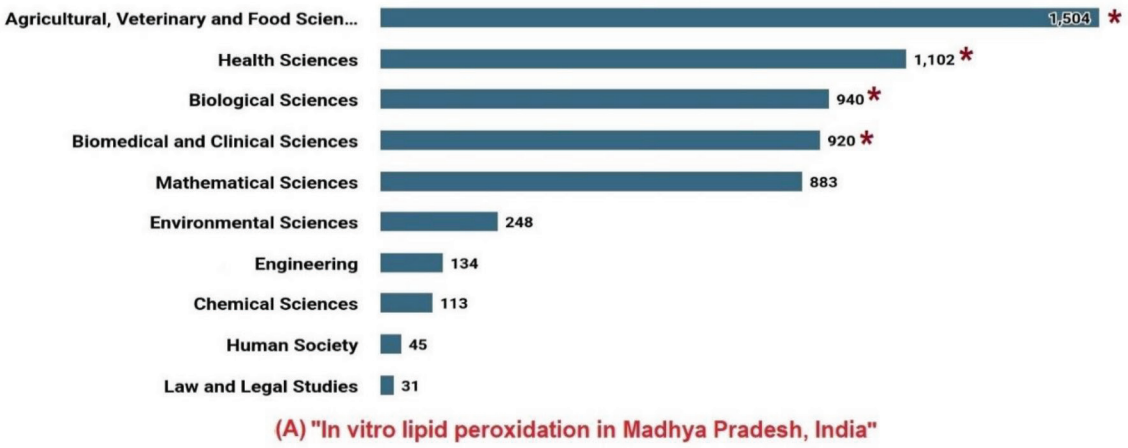




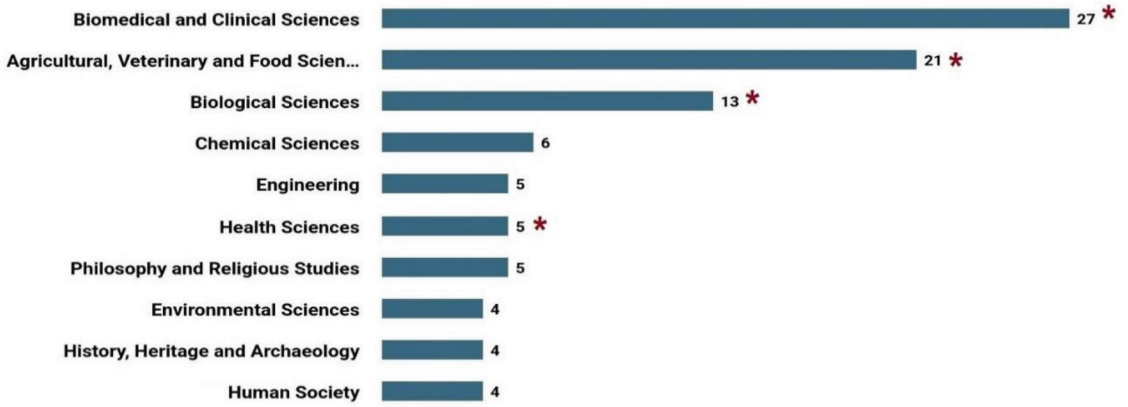


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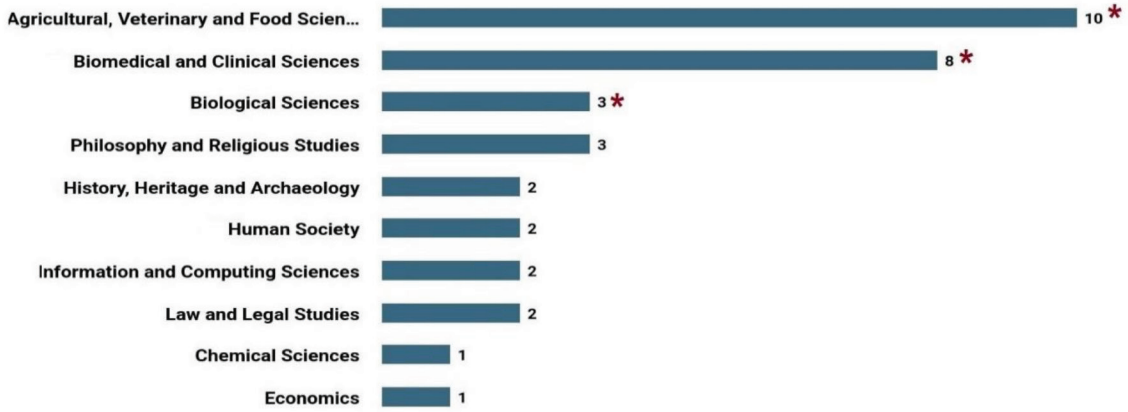
Figure 5. The bibliography of research categories with the total number of publications in each category with the keywords (a) "Lipid peroxidation", (b) "*In vitro* lipid peroxidation", and (c) "*In vitro* lipid peroxidation in India". In each case, major 10 fields of research have been mentioned above. (* indicated concern categories of publication)



(A) "In vitro lipid peroxidation in Madhya Pradesh, India"



(B) "In vitro lipid peroxidation in Guna district Madhya Pradesh, India"



(C) "In vitro lipid peroxidation of *Acacia catechu* extracts in Guna district Madhya Pradesh, India"

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Figure 6. The bibliography of research categories with the total number of publications in each category with the keywords (a) "*In vitro* lipid peroxidation in Madhya Pradesh, India", (b) "*In vitro* lipid peroxidation in Guna district, Madhya Pradesh, India", and (c) "*In vitro* lipid peroxidation in *A. catechu* extracts, Guna district, Madhya Pradesh, India". In each case, major 10 fields of research have been mentioned above. (*indicated concern categories of publication)

Table 4. Inhibition of LPO by bark extracts of *A. catechu* and standard drugs used (at 125 µg/ml sample concentration) in $FeSO_4$ (10 mM in DW) induced oxidative stress in liver and kidney tissue homogenates

Test Groups	Total LPO at 125 µg/ml sample concentration (MDA formed/hour/mg protein)	
	Liver tissue	Kidney tissue
Control	1.02±0.02	0.68±0.02
$FeSO_4$	8.49±0.32	5.83±0.02
$FeSO_4$ + Methanolic Extract	2.22±0.09	1.33±0.06
$FeSO_4$ + Ethanolic Extract	1.73±0.09*	1.11±0.05*
$FeSO_4$ + Aqueous Extract	1.84±0.09*	1.16±0.04*
$FeSO_4$ + Acetone Extract	2.02±0.11	1.2±0.061*
$FeSO_4$ + Chloroform Extract	3.16±0.11	1.93±0.06
$FeSO_4$ + Benzene Extract	3.75±0.09	2.16±0.09
$FeSO_4$ + Gallic Acid	1.34±0.04*	1.03±0.03*
$FeSO_4$ + Quercetin	1.37±0.06*	0.98±0.04*
$FeSO_4$ + Catechin	1.21±0.03*	0.81±0.02*

The * indicated comparatively equal values for LPO inhibition by sample extracts and standard drugs used.

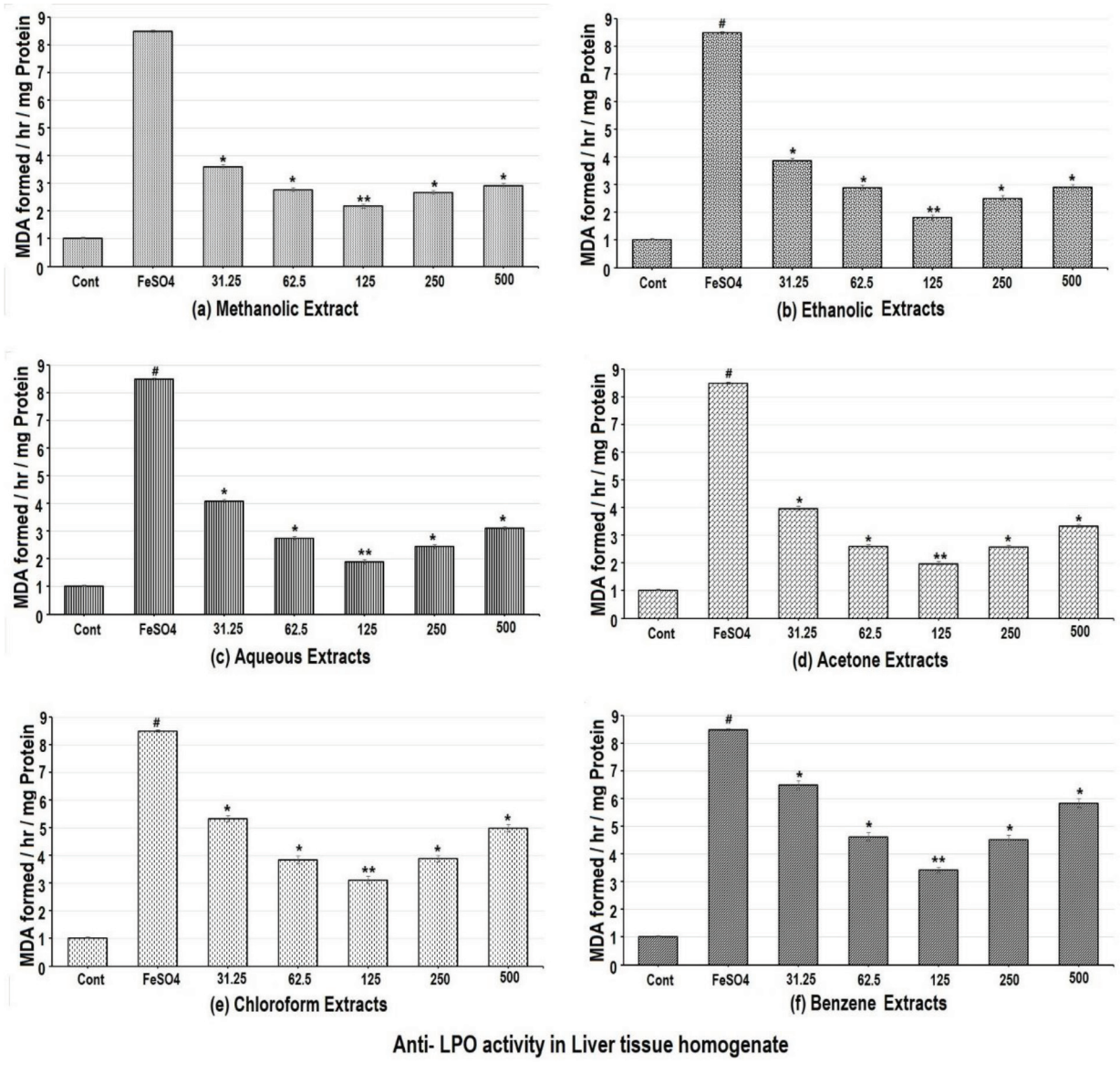


Figure 7. Comparison of concentration-dependent anti-LPO efficacy of (a) Methanolic, (b) ethanolic, (c) Aqueous, (d) Acetone, (e) Chloroform, (f) Benzene extracts of bark samples of *A. catechu* in $FeSO_4$ (10 mM in DW) induced oxidative stress in liver tissue homogenate. Drug concentrations are in µg/ml of DW.

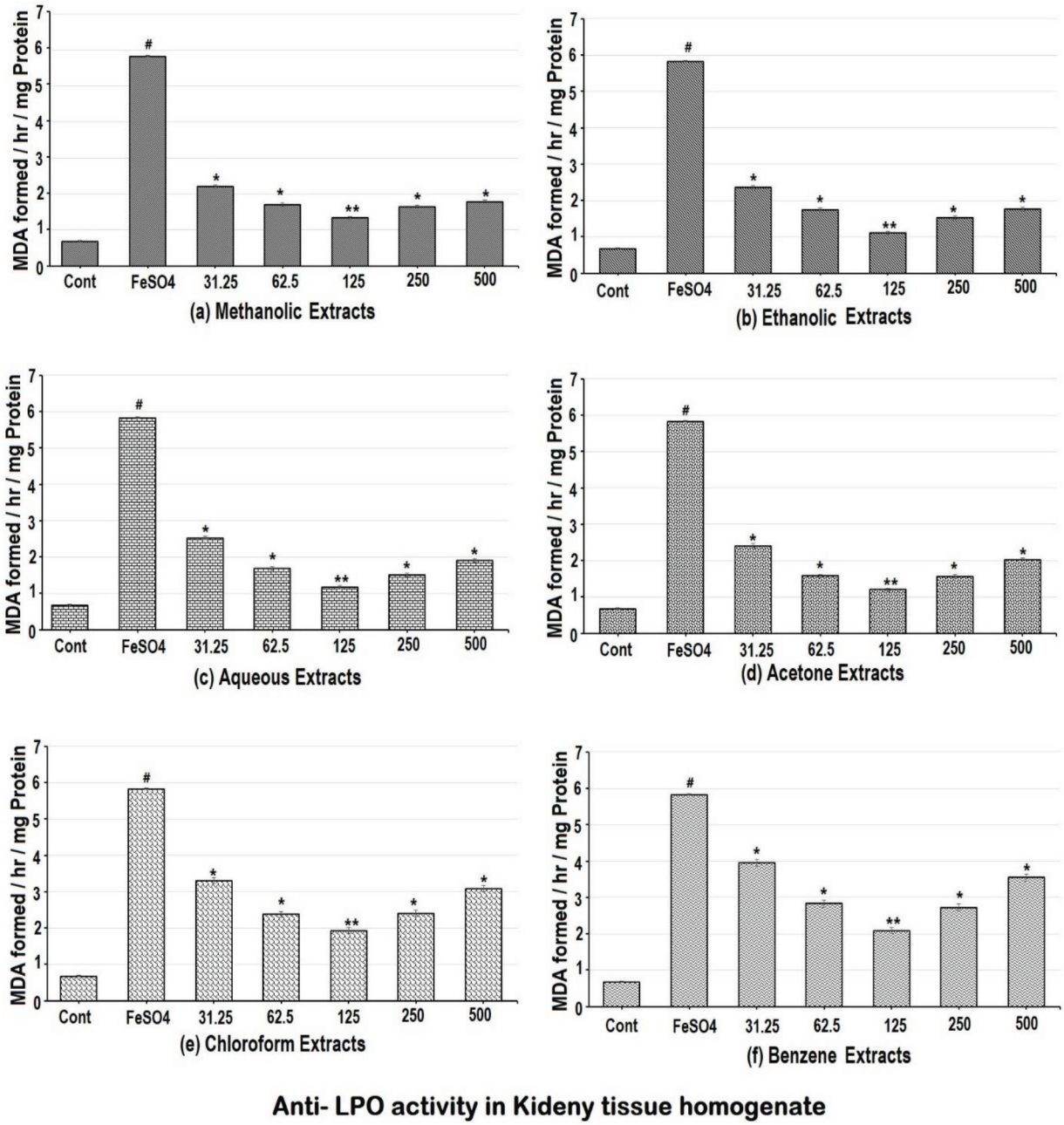


Figure 8. Comparison of concentration-dependent anti-LPO efficacy of (a) Methanolic, (b) Ethanolic, (c) Aqueous, (d) Acetone, (e) Chloroform, (f) Benzene extracts of bark samples of *A. catechu* in $FeSO_4$ (10 mM in DW) induced oxidative stress in kidney tissue homogenate. Drug concentrations are in µg/ml of DW.

research publications based on specific categories or themes within a broader field. Overall, a category-wise database enhances the comprehensiveness and usability of a bibliography, making it a valuable tool for systematic reviews and meta-analyses. Here, the number and fields of publication also provide insights into the distribution of research activity across different categories, as also reported by others[32-35].

Reactive oxygen species, also known as free radicals, are known to cause DNA damage and oxidize lipids and proteins in nearly all biological systems. Research has shown that this is the primary cause of almost all metabolic diseases, as well as issues associated with ageing[6,7,13]. Many people often view natural antioxidants as the safest and most accessible way to treat various ailments[21,28]. Therefore, possibly

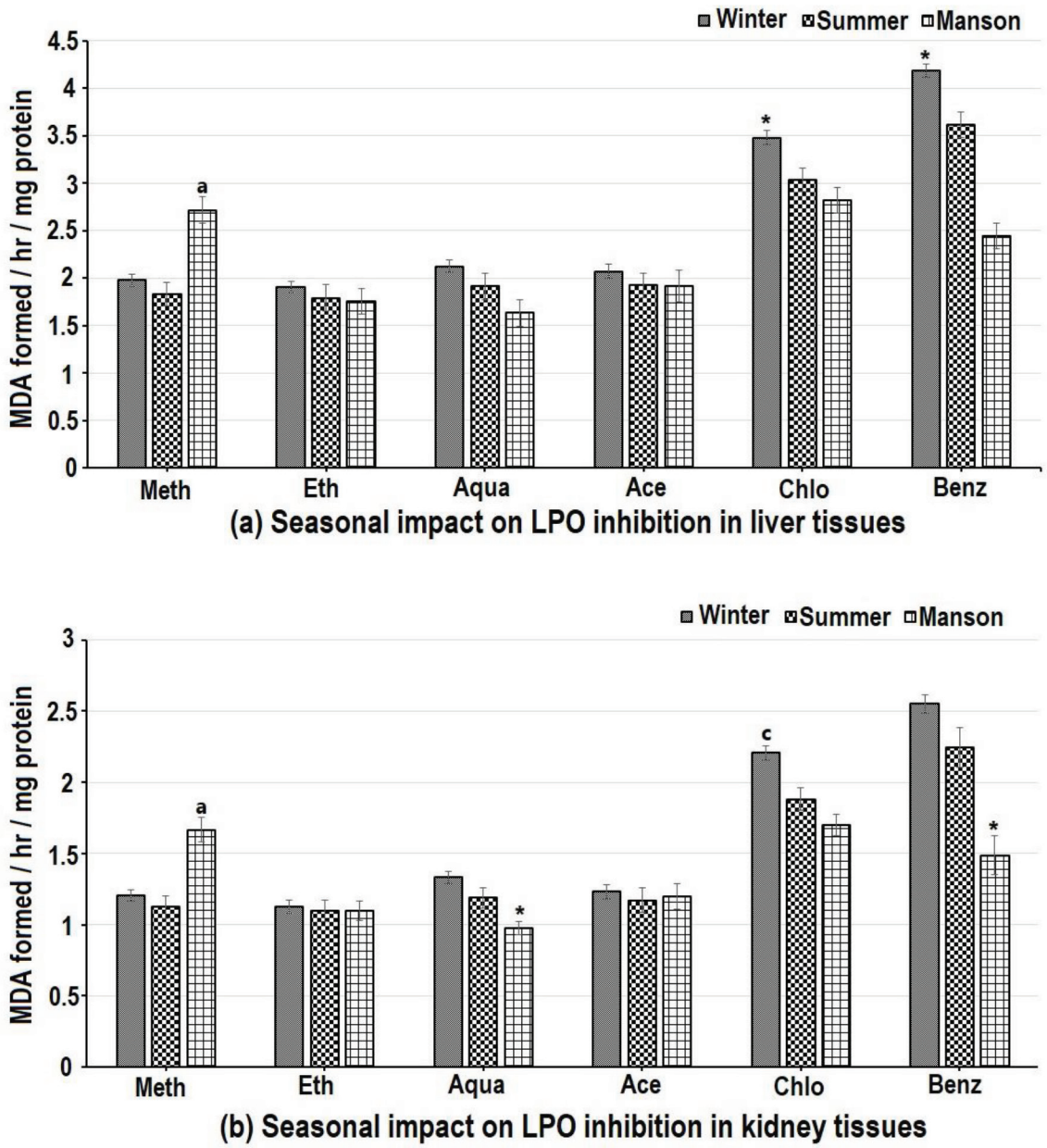


Figure 9. Comparison of anti-LPO efficacy of different bark extracts of *A. catechu* collected in different seasons in $FeSO_4$ (10 mM in DW) induced oxidative stress in (a) liver and (b) kidney tissue homogenate 125 µg/ml extract concentration.

the current results might serve as the basis for further research and might aid in meeting the need for local and accessible health supplements as well as potential medications for various disorders[29,36]. Prior research has also shown that extracts from the bark of *A. catechu* have anti-lipid peroxidative and free radical scavenging properties. Earlier reports demonstrated that the methanol and hexane extracts of *A. catechu* bark had much stronger effects on cell growth and death *in vitro* than the aqueous extracts[7,13-15,22,37].

The present investigation included liver and renal tissue homogenates because of their direct involvement in drug metabolism and susceptibility to oxidative stress. In the present study, all examined extracts demonstrated efficacy against oxidative stress induced by $FeSO_4$. Previous investigations have also confirmed these results[7,28,29,36]. Prior research has shown the defensive properties of several bark extracts derived from *A. catechu* originating in various geographical areas[16,20,31,38]. This current report, however, is the

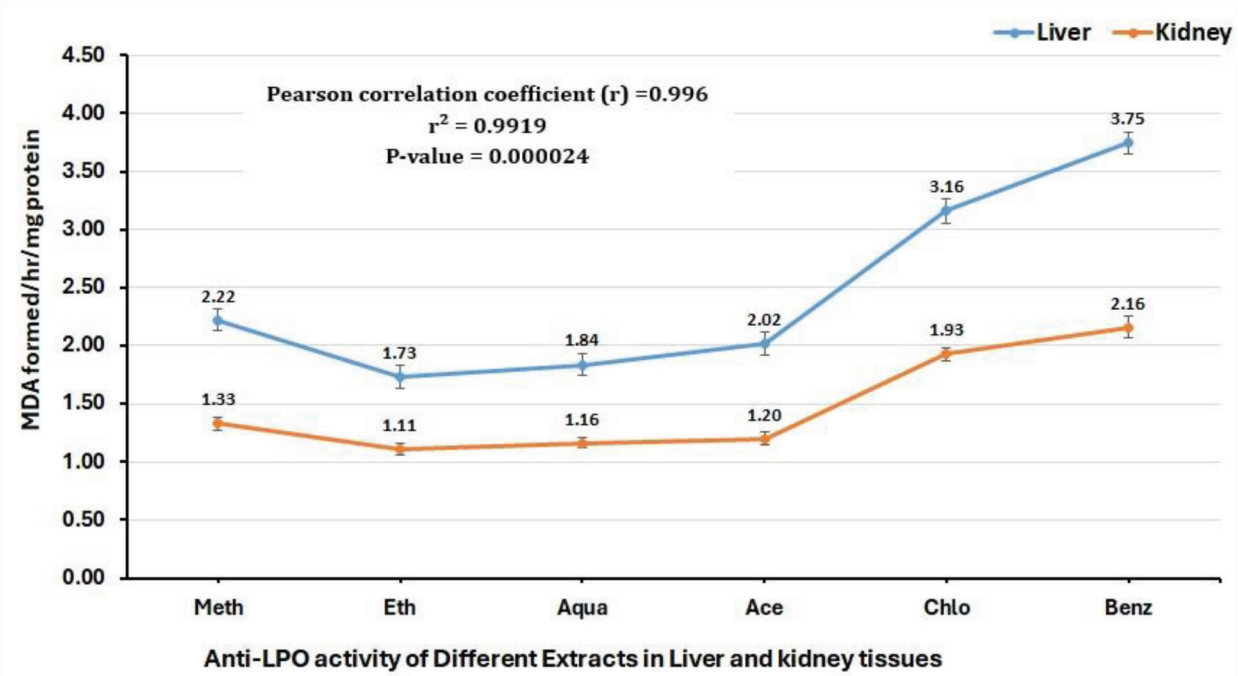


Figure 10. Comparison of anti-LPO efficacy of extracts of *A. catechu* prepared using different solvents in $FeSO_4$ (10 mM in DW) induced oxidative stress in (a) liver and (b) kidney tissue homogenate at 125 µg/ml extract concentration — the correlation analysis between the two organs mentioned above also depicted here.

first to investigate and reveal the protective action of different *A. catechu* bark extracts, specifically from the Guna region.

A number of pre-clinical and clinical studies on rats, mice, and cell cultures have shown that *A. catechu* heartwood and bark extract have antioxidative potential[2,7,13,15,19]. Moreover, the research data also suggest a close relationship between the antioxidant qualities and the anti-inflammatory, anti-neoplastic, and analgesic properties, although the exact mechanism and molecular pathways remain incompletely understood[17,35]. Furthermore, many studies have shown that catechin, the primary bioactive compound in this plant, has anticancer, analgesic, and anti-inflammatory activities due to the presence of antioxidants in the extracts. For example, studies have shown that a methanolic extract from the heartwood of a test plant can protect DNA from superoxide, nitric oxide, hydrogen peroxide, and hypochlorous acid radicals and act as an antioxidant. It can eliminate free radical generation by chelating ferrous and ferric ions[16,17,37]. Guleria *et al.,* have shown that methanol, acetone, and ethyl acetate extracts of *A. catechu* bark possess antioxidant properties and may prevent DNA strand breaks. Their findings demonstrated significant antioxidant activity and DNA preservation[20].

Furthermore, scientists have acknowledged that the test plant contains a strong chemical known as quercetin. Scientific studies have demonstrated the robust anti-inflammatory, antioxidant, and anticancer effects of quercetin[5,7,39]. Along with quercetin, catechin and epicatechin are also chief bioactive molecules of the test plant's bark extracts, which have been known for their powerful antioxidative potential[9-11,13,21,31]. The same compounds have also been known to mimic insulin, act as anti-diabetic medications, and reported to enhance cardiac function[13,17]. Additionally, the ethanolic bark extracts of *A. catechu* demonstrated anti-diabetic characteristics by effectively suppressing the activity of glucosidase and amylase enzymes. Researchers have already found that the bark of the *A. catechu* tree can increase the levels of several antioxidant enzymes, lower the damage to DNA and lipids, and raise the levels of reduced glutathione inside cells[17,22,23,40].

Along with bark extracts, the heartwood, leaf, and seed extracts of the same plant have been recognized for their antioxidant and radical-scavenging

properties[28,31,41]. Researchers have found that the methanolic extract from the heartwood of *A. catechu* arrested the growth of breast adenocarcinoma cells[8,42]. When the combination of aqueous extracts from *A. catechu* and *Rotula aquatica* were given together, they showed strong anti-cancer effects and good antioxidant activity against oxidative stress caused by cancer cells[43]. In a different study, some researchers have shown that certain polyphenolic compounds found in the bark of the same plant were better at fighting cancer than radiation therapy and were more powerful antioxidants[16,17,37]. Some workers have found that the bark of *A. catechu* had notable effects on oxidative stress and inhibited the growth of many cancer cell lines, including A549 lung, PC-3 prostate, MCF-7 breast, Hep-G2 liver, HeLa cervix, and IMR32[3,7,11,44]. Multiple studies have demonstrated the chemoprotective properties of *A. catechu* extract[4,13,20,38,41-43]. An experiment by Syed *et al.,* on male Swiss albino mice showed that both aqueous and alcohol extracts of *A. catechu* heartwood could change the immune systems of the mice[45].

Some studies have shown that when different solvent-based extracts of *A. catechu* were compared, the ethyl acetate part of the bark had the best ability to remove free radicals, beating out the water-based and other bark parts[9,21,38,40]. However, in the same experiment, the free radical scavenging capacity of various extracts varied, indicating the existence of diversity in the phytochemistry of the extracts. The examination of the test samples also detected the presence of flavonoids, tannins, and polyphenolic substances in varying quantities[46]. The presence or absence of distinct bioactive chemicals in each extract may account for the variation in the protective effectiveness of the sample extracts. The protective effects vary depending on the quantity of bioactive compounds present in the test system[5,41,43]. However, as previously documented elsewhere, higher concentrations of extracts in this study have demonstrated lesser protective effectiveness, potentially due to the occurrence of adverse drug reactions.

Evidence has demonstrated a parallel connection between the antioxidant properties and the anti-inflammatory, anti-neoplastic, and analgesic qualities. The anti-LPO capabilities of *A. catechu* bark extracts function via multiple major routes[7,47]. The extracts primarily function as potent antioxidants due to the presence of bioactive components like flavonoids, tannins, and polyphenols. These chemicals counteract the effects of free radicals, therefore inhibiting the onset of lipid peroxidation[12,30]. In addition, they can bind metal ions like iron and copper, which act as catalysts for ROS production. The extracts reduce oxidative stress by suppressing ROS generation[42]. On top of that, these antioxidants can improve the work of natural antioxidant enzymes like superoxide dismutase, catalase, and glutathione peroxidase, adding another layer of defence against lipid peroxidation[37]. Other than these, its bark extracts demonstrate antioxidative properties by inhibiting oxidative enzymes, protecting cellular biomolecules, modulating signalling pathways, and exerting anti-inflammatory effects[48]. The properties of *A. catechu* help protect against oxidative stress-related ailments, including cardiovascular diseases, neurological disorders, cancer, and inflammation. Collectively, these pathways enhance the overall effectiveness of its bark extracts in safeguarding cells against oxidative harm[26,36,39,42,48].

The influence of several seasons on the secondary bioactive constituents of *A. catechu* bark extracts is significant. Fluctuations in seasons may impact the levels and varieties of bioactive substances, such as flavonoids, tannins, and polyphenols, present in the bark[23]. In some seasons, especially when the plant undergoes stress due to fluctuations in temperature, light, and water availability, the synthesis of these chemicals may increase as a protective response[24,31]. Understanding these periodic fluctuations is critical for collecting *A. catechu* bark at the most advantageous time to maximize the level of beneficial bioactive constituents[10,21,46]. This expertise is essential to guaranteeing that the extracts obtained are of the highest therapeutic grade, providing the most significant medical advantages[17,26.49]. The current finding also revealed the seasonal impact of samples on the overall LPO inhibiting potency, which varied with the extraction mediums used. Additional research is required to fully chart these influences and devise solutions to alleviate any negative consequences.

The aforementioned experimental study has demonstrated the significant antioxidant properties of *A. catechu* bark extracts sourced from the Guna region. Future studies should concentrate on intricate mechanisms, ideal dosages, and clinical verification to guarantee their effectiveness and security for human health.

5. Conclusion

The experimental study on the antioxidative effects of plant extracts for medical uses found that these extracts, especially those from *A. catechu* bark, have a lot of potential to fight oxidative stress. Possibly, the high content of bioactive compounds such as flavonoids, tannins, and polyphenols in these extracts contributes to their strong antioxidant properties[3,7,13,31,37]. The findings suggest that these plant extracts could be valuable in developing natural antioxidant therapies for preventing and treating diseases associated with oxidative damage[22,28,34,45]. The present preliminary study supports the use of plant extracts as natural antioxidant agents in medicinal applications, providing a promising alternative to synthetic antioxidants. Further investigation is required to clarify the precise mechanisms of action, optimize doses, and carry out clinical studies to verify their safety and effectiveness in situations involving human health. Additional research is recommended to explore their full therapeutic potential and application in clinical settings.

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