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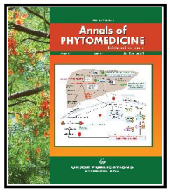

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Phytochemical analysis, antioxidant and DNA nicking protection assay of some selected medicinal plants

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Abstract

The abundance of metabolites from plants exhibits diverse chemical structures and potentially possess a variety of effects like therapeutic values which have been documented in many traditional systems of medicines. The formulations in the Unani medicine systems are based on these health benefits of the plants for the treatment of diseases. Osteoarthritis (OA) is a worldwide illness that is chronic and incapacitating. This study was planned with the objective to examine the phytochemical components of some selected plants' aqueous extracts used in the Unani system of medicine to treat osteoarthritis. These plants include *Aloe barbadensis*, *Chrysanthemum indicum*, *Commiphora mukul*, *Convolvulus scammonia, Ipomoea turpethum*, and *Merendera persica*. Established methods like Bradford, DNSA, Folin Ciocalteau's and Aluminum chloride were employed to assess the protein, reducing sugar, total phenolic, and flavonoid content present in the aqueous extracts of the above-mentioned plants, respectively. The antioxidant activities of the aqueous extracts were determined using the 2,2-diphenyl-1-picrylhydrazyl, 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonate), nitric oxide assay and ferric reducing antioxidant power assays. The aqueous extract of most of the plants demonstrated considerable antioxidant activity, with *M. persica* showing highest radical scavenging activity. However, there was a good correlation between the phytochemicals and antioxidant activity for the *A. barbadensis*. The DNA nicking prevention studies indicate the better potential of *M. persica*, *A. barbadensis* and *C. scammonia* among the plants tested. These results indicate the potential health benefit especially prevention of oxidative stress which is implicated in many disease conditions like diabetes, cancer, osteoarthritis, *etc*.

1. Introduction

Osteoarthritis (OA) stands as the most widespread type of arthritis and joint disorder on a global scale. The disease advances, the person's ability to work is impacted, which causes dependency and separation from a productive life (Tarannum *et al.*, 2016). OA is thus becoming a significant factor in lost employment. Controlling the pain, enhancing mobility, and reducing morbidity are the main objectives in the management of OA. Old age, chronic illnesses, congenital organ weakness, emotional disturbances, alcoholism, insomnia, sedentary lifestyle, women's menstrual irregularities, trauma, heredity, and other factors are exciting (Khatoon *et al.,* 2023). To treat OA, Unani practitioners have traditionally used medications like *Asarum europeaum, Apium graveolens, Piper longum, Matricaria chamomilla*, and *Butea frondosa* are included. These medications have analgesic, anti-inflammatory, nerve tonic, muhallil, and diuretic properties, respectively.

Plants, a precious gift from nature, provide not just food but also vital medicinal properties for preventing and treating various ailments (Basha and Sudarshanam, 2010). Ayurveda, Siddha, and Homeopathy exemplify medical traditions that harness these benefits (Ali, 2020). This traditional knowledge has been passed down through generations in regional cultures (Ajazuddin and Saraf, 2012). The World Health Organization (WHO) encourages member nations to promote traditional medicine for both prevention and treatment. In resource-constrained countries like India, modern allopathic therapy can be financially burdensome and inaccessible in remote or underserved areas due to a shortage of contemporary doctors (Borins, 1987). The search for alternatives to current products is therefore ongoing, and natural phytochemicals, chemicals derived from plants and used in traditional medicine. are seen as promising replacements for synthetic chemicals (Tichy and Novak, 1998).

Colchicine, an alkaloid present in plant seeds and corms, serves as a remedy for pain and inflammation (Hassan *et al.,* 2021; Sánchez *et al*., 2019). It also facilitates chromosome doubling in plant breeding. Aloe, with a rich history, is utilized for alleviating redness, injuries, and inflammation (Sánchez *et al*., 2020). The dried flower heads of diverse *Chrysanthemum* species have played a prominent role in Chinese and Ayurvedic medicine (Youssef *et al.,* 2020). *Ipomoea turpethum*, utilized in Ayurvedic medicine, addresses a spectrum of conditions like vitiligo, induced lacrimation, cervical lymphadenitis, chronic gout, constipation, fever, bronchitis, ulcers, hemorrhoids, tumors (Gupta and Ved, 2017). *Convolvulus scammonia*, commonly employed as a purgative, aids in eliminating morbid humours, par-

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ticularly bilious humour, associated with various diseases. Unani doctors note potential negative side effects including agitation, melancholy, palpitations, nausea, reduced appetite, and increased thirst. Caution is advised, as it may have adverse effects on the heart, stomach, and liver (Ansari *et al.,* 2022).

Guggul, when consumed orally, is utilized in Ayurvedic medicine to treat obesity, malignant sores, internal tumors, liver issues, intestinal worms, leucoderma, sinus problem, and edema. Moreover, it is utilized to treat other conditions such as diabetes, inflammatory bowel disease, ulcers, arthritis, cardiovascular diseases, as well as their prevention (Kunnumakkara *et al.,* 2018). This paper presents the phytochemical analysis, antioxidant activity and DNA damaging studies of the selected plants that include *Aloe barbadensis, Chrysanthemum indicum*, *Commiphora mukul, Convolvulus scammonia, Ipomoea turpethum, and Merendera persica*.

2. Materials and Methods

2.1 Procurement and extraction

The plants were procured from Unani sample suppliers and authenticated by Dr. Mohammad Rashid (Department of Saidla, Faculty of Unani Medicine, Ajmal Khan Tibbia College, Aligarh Muslim University) with taxonomic recognition (Certificate No. 843/DS). The aqueous extracts were prepared using Soxhlet extraction (5 g) of powdered plants and solvents that were separated at a 1:10 (w/v) ratio. The water was removed and only the dry extract stored at 4°C for further use.

2.2 Phytochemical screening of extracts

The extracts were evaluated using reported methods for various phytoconstituents like alkaloids, carbohydrates, phenols, steroids, flavonoids, proteins, glycosides, and tannins. All these phytochemical tests were done on aqueous extract on these plants (Shaikh and Patil, 2020). The powder obtained was weighed, and the yield was determined using the formula:

Percentage yield = (Actual yield/Theoretical yield) × 100
(Khandelwal, 2006)

2.3 Quantitative analysis of phytochemicals

2.3.1 Protein content

The total protein content of the plant extracts was assessed utilizing the Bradford method (Bradford, 1976). A positive control using BSA (0-1 mg/ml) was employed, and the absorbance was measured at a wavelength of 595 nm.

2.3.2 Reducing sugars content

The method described by Gusakov *et al.* (2011) was modified in order to determine the quantity of reducing sugar. To determine the level of reducing sugar present in the plant extracts, D-glucose (0-1 mg/ml) was used as a reference.

2.3.3 Total phenolic content (TPC)

The total phenolic content of the plant extracts was determined using the Folin-Ciocalteu method (Lu *et al.,* 2011). This involved adding 2.5 ml of 10% FC reagent (v/v) to 500 µl of the extract (1 mg/ml). After 5 min, 2.5 ml of 7.5% sodium carbonate (w/v) was introduced, mixed thoroughly, and allowed to incubate for 45 min at room temperature. Following incubation, the absorbance was measured at 765 nm using a Shimadzu UV-1800 spectrophotometer, with a blank (lacking extract) serving as the reference. To create a calibration curve, gallic acid (5-500 mg/l) was employed, and milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g DW) was used to express the results.

2.3.4 Total flavonoid content (TFC)

The aluminum chloride colorimetric method was used to assess the total flavonoid content in the extracts of plants (Shraim *et al.,* 2021). In conclusion, 2.5 ml of distilled water, 1 ml of 1 M potassium acetate, and 0.5 ml of the plant extract (1 mg/ml) were combined. The absorbance of the mixtures was measured at 415 nm using a UV spectrophotometer. A calibration curve was established within the range of 0-50 µg/ml using quercetin, and the findings were presented as milligrams of quercetin equivalent per gram of dry weight (mg QE/g DW).

2.4 Antioxidant activity of extracts

The antioxidant potential of six aqueous extracts was assessed through DPPH, ABTS, Nitric oxide, and FRAP assays.

2.4.1 The DPPH assay

The DPPH method was utilized to evaluate the radical scavenging potential of each sample (Baliyan *et al.,* 2022). The process involved mixing 500 µl of 0.3 mM DPPH (dissolved in methanol) with 500 µl of varied extract concentrations, spanning from 200 to 1000 µg/ml. After incubation in darkness for 30 min, absorbance was measured at 517 nm, using methanol as a blank and DPPH-methanol mixtures as controls. Ascorbic acid served as the positive control. The percentage of inhibition was calculated using this formula: (AC - AS/AC) × 100%, where AC represents the absorbance of the control (without extract), and AS represents the absorbance of the sample (with extract).

2.4.2 The ABTS assay

The ABTS assay was used to evaluate the capacity to scavenge free radicals (Lalhminghlui and Jagetia, 2018). In summary, 250 µl different concentrations of plant extracts (ranging from 200 to 1000 µg/ml) were mixed with the $ABTS^{+}$ solution (750 µl) and incubated for 10 min. The absorbance was measured at 734 nm, with gallic acid serving as the positive control. The inhibition percentage was determined using the formula: (AC - AS/AC) × 100%, where AC stands for the absorbance of the control sample (lacking the extract), and AS denotes the absorbance of the sample with the extract.

2.4.3 NO assay

Each sample's nitric oxide scavenging assay was identified. Different concentrations of the extract were mixed with sodium nitroprusside (10 mM) in PBS (0.02 M, pH 7.4) employing appropriate solvents (Das *et al.,* 2012). The mixture was then incubated at 25°C for an hour. For the control, an equivalent reaction mixture was made, substituting the extract with an equal volume of ethanol. After incubation, 1.5 ml of the solution was taken and combined with 1.5 ml of Griess reagent, containing 0.1% naphthyl ethylenediamine dihydrochloride, 1% sulfanilamide, and 20% glacial acetic acid. Using sulfanilamide to diazotize the nitrite and then coupled with naphthyl ethylenediamine, a chromophore was produced that had an absorbance of 546 nm.

2.4.4 Ferric reducing antioxidant potential (FRAP) assay

The FRAP solution was prepared using 10 mM of 2,4,6-Tris (2-pyridyl)-1,3,5-triazine (TPTZ) dissolved in 40 mM HCl, combined with 20 mM $FeCl_3$ in water and 300 mM acetate buffer (pH 3.6) in a proportion of 1:1:10 (Apak *et al.,* 2018). To correct the color, a blank containing only the sample and solvents was utilized, and the absorbance was assessed at 600 nm. Trolox were employed as antioxidant standards and positive controls.

2.4.5 DNA nicking protection assay

pBR322 plasmid DNA (0.25 g), H_2O_2 (60 mM), were incubated in 100 mM phosphate buffer (pH 7.4) with and without plant extracts to study the H_2O_2-mediated DNA damage (Ali *et al.,* 2022). Sample, including pBR322, pBR322+H_2O_2, pBR322 and the extract underwent incubation at 37°C. Subsequently, the samples were segregated using a 1% agarose gel post-incubation, followed by visualization using Gel-Doc was done.

2.5 Statistical analysis

The results are expressed as mean ± SD (n = 3), and Tukey's multiple comparison tests were conducted to determine differences among treatments, with significance set at $p<0.05$ compared to the positive control.

3. Results

3.1 Extractive values of extracts

Table 1 provides the physicochemical characteristics of plants. The aqueous extract *C. mukul* exhibited good percent yield of 44.60 ± 0.43 w/w while that of *C. scammonia* was found to be the lowest among all the plants.

Table 1: Percentage yield of extracts

S. No.	Plant	Extractive value (% yield)
1.	*C. indicum*	25.40 ± 0.10
2.	*C. mukul*	44.60 ± 0.43
3.	*C. scammonia*	14.90 ± 0.10
4.	*I. turpethum*	24.53 ± 0.07
5.	*M. persica*	26.33 ± 0.07

3.2 Qualitative analysis

The results of the phytochemicals revealed the presence of phenols, tannins, flavonoids, terpenoids, steroids, saponins, and carbohydrates to be more in *A. barbadensis* and *M. persica*, while *C. indicum* exhibited comparatively a smaller number of phytochemicals (Table 2).

3.3 Quantitative analysis of extracts

3.3.1 Carbohydrate and protein content

The results of carbohydrate content assays indicated *C. indicum* gives lowest content (1.07 ± 0.08 mg/ml) and *A. barbadensis* gives highest content (6.23 ± 3.32 mg/ml) as shown in Table 3. The assays for the protein content revealed that *C. mukul* (0.13 ± 0.00 mg/ml) had the lowest content, while *A. barbadensis* exhibited the highest content (0.58 ± 0.03 mg/ml) as represented in Table 3. These quantitative values indicate that *A. barbadensis* contains high amount of reducing sugar as well as protein compared to other plants.

Table 2: Phytochemical investigations of selected plant extracts

S.No.	Phytochemicals	*A. barbadensis*	*C. indicum*	*C. mukul*	*C. scammonia*	*I. turpethum*	*M. persica*
1	Carbohydrates	+	+	+	+	+	+
2	Amino acid and proteins	+	-	-	-	+	+
3	Steroids	-	-	-	-	-	-
4	Glycosides	-	-	+	-	-	+
5	Flavonoids	+	-	-	-	-	-
6	Phenols	-	+	+	+	+	+
7	Tannins	+	-	-	-	-	-
8	Terpenoids	+	+	+	+	+	+
9	Alkaloids	+	-	-	+	-	+

(+) indicates presence; (-) indicates absence

Table 3: Total carbohydrate and protein content

Plant	Total carbohydrate content (mg/ ml)	Protein content (mg/ml)
A. barbadensis	6.23 ± 3.32	0.58 ± 0.03
C. indicum	1.07 ± 0.08	0.33 ± 0.02
C. mukul	1.47 ± 0.08	0.13 ± 0.00
C. scammonia	2.36 ± 0.15	0.40 ± 0.01
I. turpethum	1.37 ± 0.09	0.15 ± 0.00
M. persica	3.28 ± 0.16	0.37 ± 0.01

3.3.2 Total phenolic content (TPC)

The phenolic content assays (Table 4) indicated that *C. mukul* had the lowest content (0.58 ± 0.16 mg of GAE/g DW), whereas *A. barbadensis* showed the highest content (74.83 ± 2.75 mg of GAE/g DW). *C. scammonia* also contained comparatively high phenolic content as compared to other plants.

3.3.3 Total flavonoid content (TFC)

Similar to other phytochemicals reported in previous sections *A. barbadensis* demonstrated the highest flavonoid content (730.08 ± 19.54 mg QE/g DW) while *M. persica* exhibited the lowest amount (0.50 ± 0.02 mg QE/mg DW) as shown in Table 5.

Table 4: Total phenolic content

Plant	Total phenolic content(mg equivalent gallic acid)
A. barbadensis	**74.83 ± 2.75**
C. indicum	1.20 ± 0.02
C. mukul	0.58 ± 0.16
C. scammonia	11.25 ± 0.69
I. turpethum	2.85 ± 0.08
M. persica	1.76 ± 0.05

Table 5: Total flavonoid content

Plant	Total flavonoid content (mg equivalent quercetin)
A. barbadensis	730.08 ± 19.54
C. indicum	8.00 ± 0.60
C. mukul	27.71 ± 1.16
C. scammonia	6.87 ± 0.16
I. turpethum	9.8 ± 0.97
M. persica	0.50 ± 0.02

3.4 Antioxidant assays

3.4.1 DPPH assay

This assay assesses the ability of the test sample to donate hydrogen atoms, which is indicated by the change in color of an antioxidant compounds cause the methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) to shift its color from violet/purple to yellow. The scavenging potential of both the aqueous extract and the standard ascorbic acid demonstrated a dependency on concentration, as shown in Figure 1. All extracts exhibited antioxidant activity at par with ascorbic acid. The aqueous extract of *C. indicum* exhibited the highest IC_{50} value (5.38 µg/ml). On the other hand, *M. persica*'s aqueous extract displayed the lowest IC_{50} value (2.30 µg/ml) among all plants.

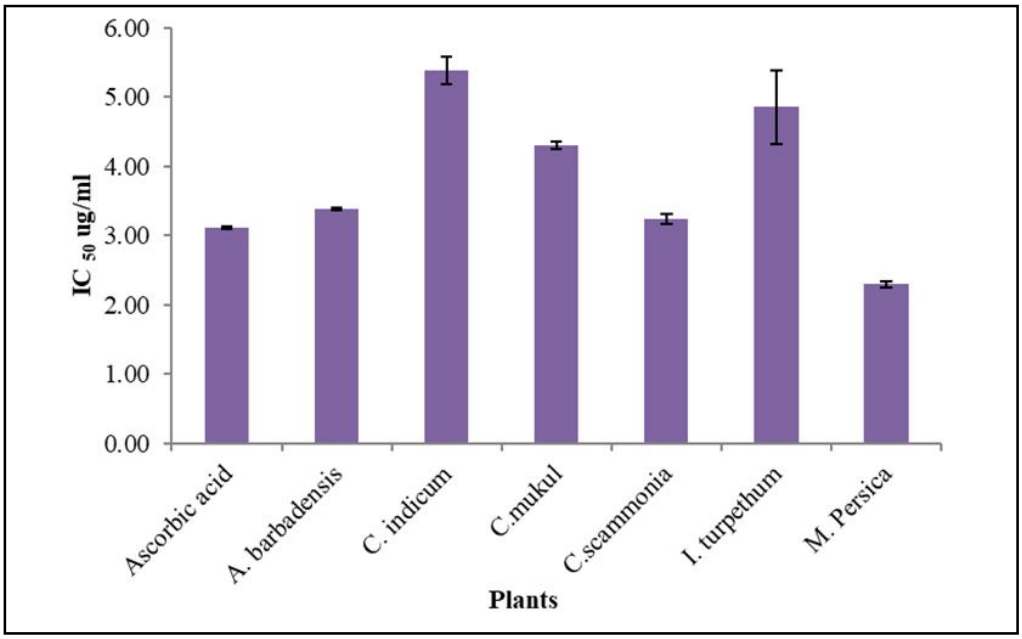

Figure 1: The DPPH assay of selected plants. Values are expres-sed as the mean ± SD (n = 3), p<0.05 when compared to ascorbic acid.

3.4.2 ABTS assay

The ability of ABTS to counteract radical cations suggests its potential to furnish hydrogen. Phenolics with higher molecular weights exhibit a more effective ability to suppress free radicals ($ABTS^{\bullet+}$). As per the Figure 2 among the plant extracts, *C. mukul* displayed the highest value (3.12 µg/ml) for scavenging ABTS while *C. scammonia* demonstrated the lowest IC_{50} value (0.68 µg/ml). Therefore, the extracts exhibited a higher potency compared to the standard gallic acid.

3.4.3 NO assay

Nitric oxide produced from sodium nitroprusside reacts with oxygen, resulting in the formation of nitrite. The assessment of nitric oxide radical scavenging was conducted by generating nitric oxide from sodium nitroprusside in buffered saline. This produced nitrite ions,

which were quantified using Griess reagent. Figure 3 exhibits antioxidant activity of the plants which were found to be higher than that of gallic acid. As can be observed in the figure *C. indicum* showed the highest IC_{50} value (6.33 µg/ml) while *M. persica* aqueous extract displayed the lowest IC_{50} value (4.18 µg/ml) among all the plants.

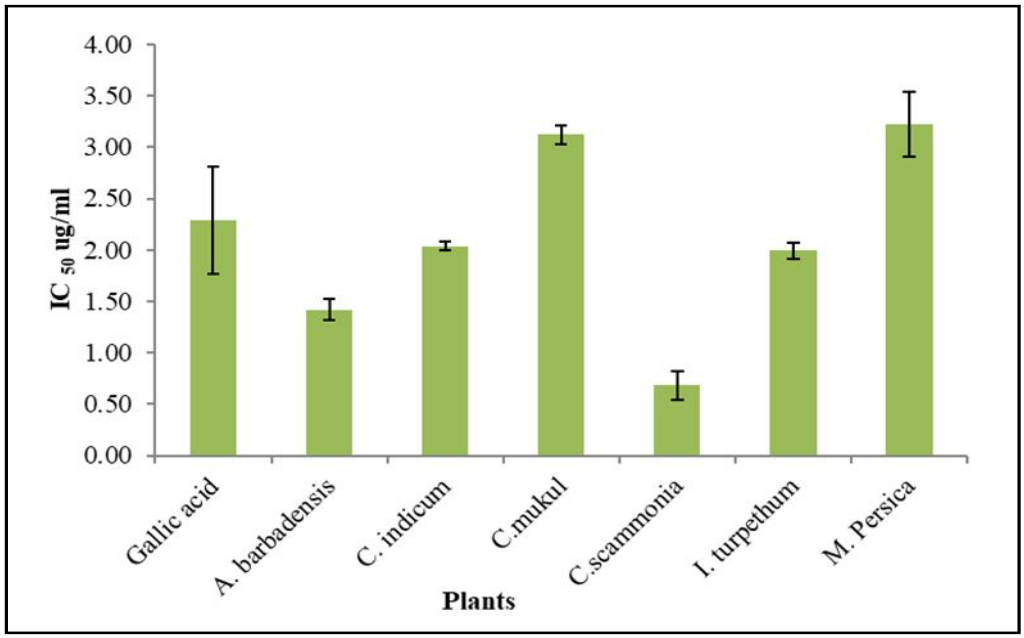

Figure 2: The ABTS assay of selected plants. Values are expressed as the mean ± SD (n = 3), $p<0.05$.

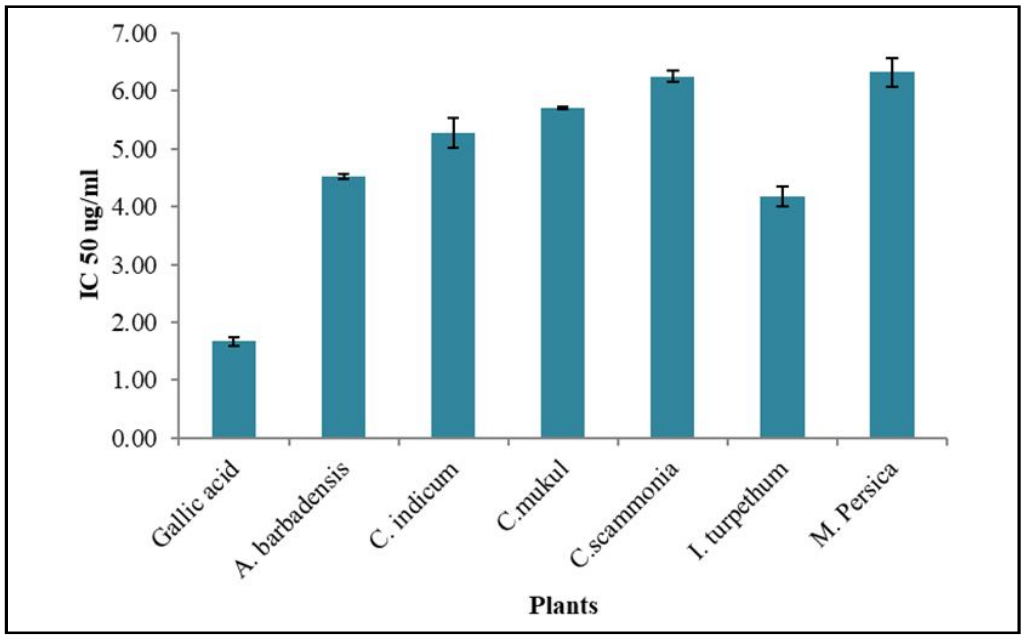

Figure 3: The NO assay of selected plants. Values are expressed as mean ± SD (n = 3) and a significance level of $p<0.05$.

3.4.4 The FRAP assay

The FRAP assay assesses an antioxidant's ability to reduce a ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex, generating a colored ferrous tripyridyltriazine (Fe^{2+}-TPTZ). This antioxidative function involves disrupting neutralizing the free radical chain is achieved by providing a hydrogen atom. At a low pH around 3.6, the Fe^{3+}-TPTZ complex undergoes reduction, producing a blue-colored Fe^{2+}-TPTZ compound. The antioxidant activity, evaluated via the FRAP assay, escalates with increasing extract concentration. Among them, *I. turpethum* displays the highest antioxidant potential, while *C. indicum* exhibits the lowest. All extracts exhibit a comparable effect to Trolox (Figure 4).

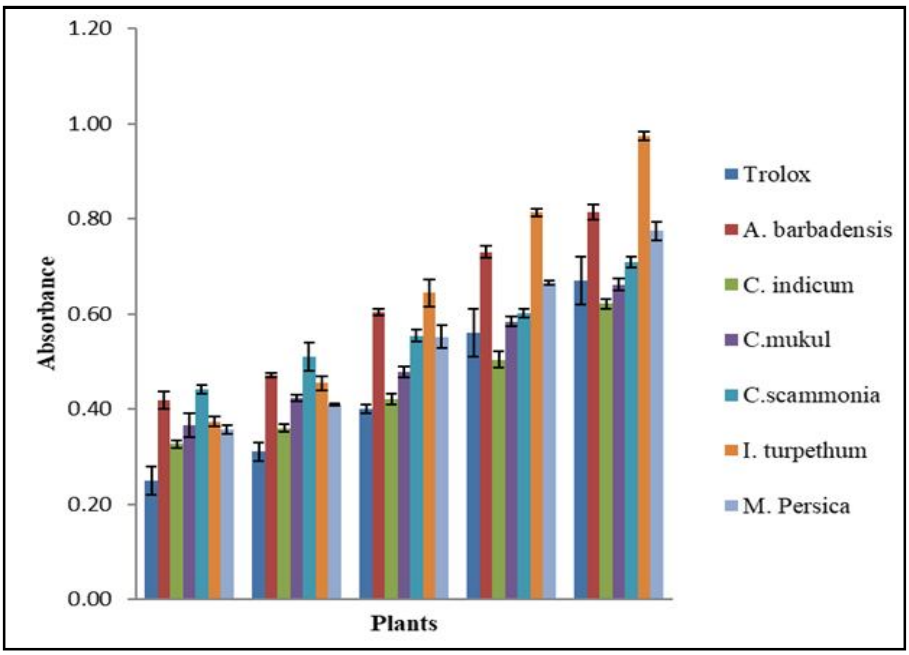

Figure 4: The FRAP assay of the selected plants. Values are expressed the mean ± SD (n = 3) with a significance level of $p<0.05$.

3.4.5 DNA nicking protection assay

The results of DNA damage indicate that Figure 5 (a) and (b) in Lane 1, Control DNA was found with two distinct bands: one demonstrated open circular DNA, while the other presented supercoiled DNA. In the absence of the plant extract (Figure 5a, Lane 2), treating pBR322 DNA with H_2O_2 caused the fragmentation of the supercoiled DNA, resulting in the generation of open circular DNA. In Figure 5(a), the presence of plant extracts (Figure 5a, Lanes 3-10), this breakage of strands was halted/prevented. Notably, the aqueous extracts of *A. barbadensis* and *I. turpethum* (Figure 5a, Lanes 6 and 10) exhibited the highest efficacy in averting DNA damage compared to the (Figure 5a, Lanes 4 and 8) extracts, respectively. When plant extracts of *M. persica* and *C. indicum* were present (Figure 5b, Lanes 4 and 6), they effectively prevented this strand scission. Notably, the aqueous extract of *C. scammonia*, specifically in Lane 6 (Figure 5b), demonstrated the highest effectiveness in safeguarding against DNA damage.

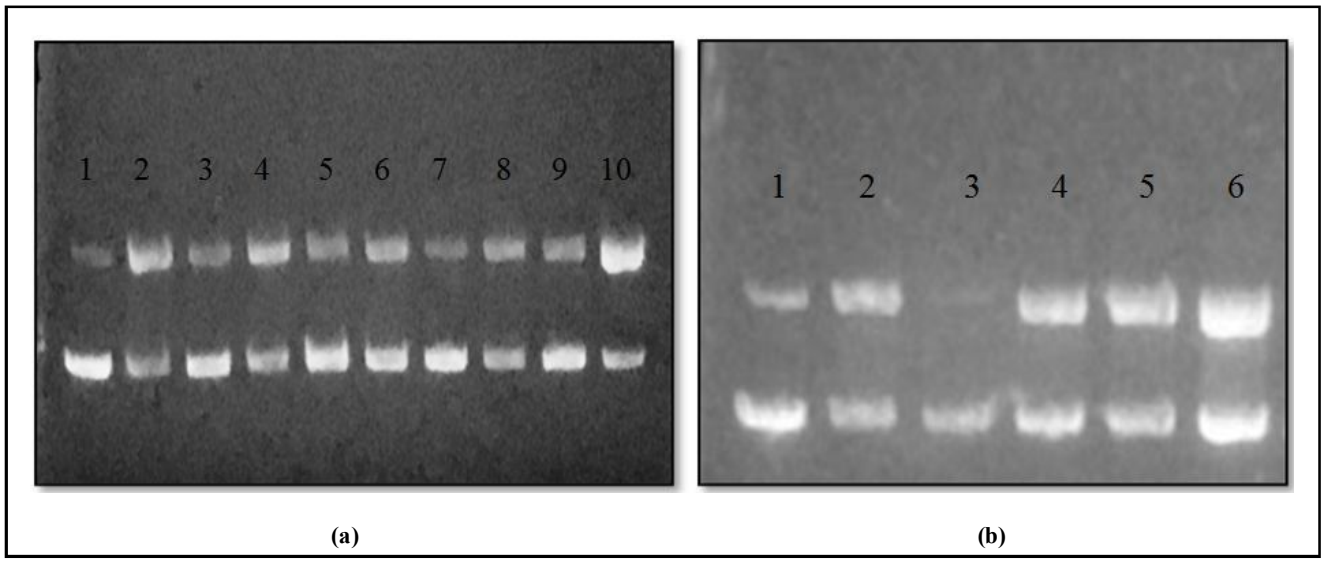

Figure 5: DNA nicking protection assay for selected plants . (a) Lane 1: control DNA; Lane 2: DNA + H_2O_2; Lane 3: DNA + *M. persica*; Lane 4: DNA + H_2O_2 + *M. persica*; Lane 5: DNA + *A. barbadensis*; Lane 6: DNA+ H_2O_2 + *A. barbadensis*; Lane 7: DNA+ *C. indicum*; Lane 8: DNA+ H_2O_2 + *C. indicum*; Lane 9: DNA+ *I. turpethum*; Lane 10: DNA+ H_2O_2 + *I. turpethum* (b) Lane 1: control DNA; Lane 2: DNA + H_2O_2; Lane 3: DNA + *C. mukul*; Lane 4: DNA + H_2O_2 + *C. mukul*; Lane 5: DNA + *C. scammonia*; Lane 6: DNA+ H_2O_2 + *C. scammonia*.

4. Discussion

Osteoarthritis is a chronic joint condition prevalent worldwide, impacting a substantial percentage of the population. It primarily involves the degeneration of joint cartilage and bone, resulting in pain, stiffness, and reduced mobility. Oxidative stress and inflammatory responses are contributory factors in this disease condition. Several approaches have been used to treat the conditions arising due to osteoarthritis. However, the anticipated success has not been achieved. In literature, there are reports about the efficacy of medicinal plants in suppressing this and other inflammatory conditions has been reported in the traditional systems of medicine like Ayurveda, Unani and Chinese. In the present study, some plants used in Unani medicine has been analyzed for their antioxidant potentials using radical scavenging tests and DNA nicking protection assays.

The aqueous extract of *C. mukul* demonstrated a higher yield, although it contained lower levels of protein and phenolic content compared to *A. barbadensis* (Kunnumakkara *et al.,* 2018). Conversely, *A. barbadensis* exhibited higher carbohydrate, protein, phenolic, and flavonoid content which is similar to earlier reports (Beppu *et al*., 2003). The examination of these plant extracts is significant due to their potential therapeutic implications for OA management. The higher nutritional and bioactive compound content in *A. barbadensis* suggests a richer source of compounds with anti-inflammatory and antioxidant properties, which are crucial for combating the oxidative stress and inflammation often associated with OA.

The presence of phenols, tannins, flavonoids, terpenoids, steroids, saponins, and carbohydrates in the phytochemical screening of these plant extracts indicates a diverse array of active compounds within them (Matsuura and Fett-Neto, 2015). Among these extracts, *A. barbadensis* had a significant content, showcasing significant levels of phenols and flavonoids. These compounds are renowned for their antioxidant and anti-inflammatory properties (Tungmunnithum *et al.,* 2018). Phenols, a group of organic compounds, are recognized for their antioxidative potential. They play a pivotal role in neutralizing free radicals and reducing oxidative stress in the body. Flavonoids, another class of bioactive compounds, possess strong antioxidant and anti-inflammatory effects. They aid in minimizing inflammation and shielding the body against oxidative damage, which are critical factors in the pathogenesis of osteoarthritis (Zhu *et al.,* 2018). The lower content of phenols and flavonoids in *C. mukul* may indicate a lesser antioxidant potential compared to *A. barbadensis.* Antioxidants play a crucial role in scavenging free radicals, which are implicated in the progression of OA by causing oxidative damage to joint tissues (Ozgen *et al.,* 2010).

The disparity in the presence of these bioactive compounds among different plant extracts highlights the variability in their potential therapeutic efficacy against OA. The high phenolic and flavonoid content in *A. barbadensis* may suggest a greater ability to alleviate oxidative stress and inflammation associated with OA (Ahmad, 2010).

The assays collectively underscore the robust antioxidant potential of the plant extracts, notably *M. persica* and *I. turpethum*. Their ability to counteract oxidative stress and protect against DNA damage suggests their potential therapeutic value in managing oxidative stress-related conditions (Balyan and Ali, 2022). To comprehend their mechanisms and validate their applications in addressing oxidative stress-related conditions, additional in-depth research is warranted. This research would help to elucidate the precise pathways through which these extracts exert their therapeutic effects and their specific applications in managing conditions associated with oxidative stress. Further research is needed to understand their mechanisms and validate their applications in addressing such conditions.

5. Conclusion

Arthritis, a prevalent issue among older individuals, affects nearly one-fifth of the global population. Treating arthritis and related inflammatory conditions typically involves a range of medication types such as non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and disease-modifying anti-rheumatic drugs. However, NSAIDs' usage is linked to gastrointestinal side effects, affecting patients' adherence to treatment. *A. barbadensis* extracts have the highest levels of polyphenols followed by *C. mukul* extracts have the lowest levels. Flavonoid content of *A. barbadensis* was significantly better when compared to other extracts and *M. persica* have least value. Most of the plants showed that very high antioxidant potential as compared to the reference standards used in the respective methods. The varying compositions of phytochemicals in these plant extracts warrant further investigation into their specific mechanisms of action against osteoarthritis. *A. barbadensis,* with its notable phenolic and flavonoid content, emerges as a promising candidate for potential use in osteoarthritis management. However, comprehensive studies, including *in vivo* trials and clinical evaluations, are imperative to establish their effectiveness and safety for treating osteoarthritis. Therefore, the objective of this study was to assess the legitimacy of their traditional applications.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

Ahmad, B. (2010). Antioxidant activity and phenoliccompounds from *Colchicum luteum* Baker (Liliaceae). Afr. J. Biotechnol., **9**(35):5762-4766

Ali, A. (2020). Herbs that heal: the philanthropic behavior of nature. Ann. Phytomed., **9**(1):7-17.

Ali, A.; Shahu, R.; Balyan, P.; Kumari, S.; Ghodmare, R.; Jobby, R. and Jha, P. (2022). Antioxidation and antiglycation properties of a natural sweetener: *Stevia rebaudiana*. Sugar Tech., **24**:563-575 https://doi.org/10.1007/s12355-021-01023-0..

Ajazuddin and Saraf, S. (2012). Legal regulations of complementary and alternative medicines in different countries, Pharmacog. Rev., **6**(12):154-160.

Ansari, H.; Ansari, A.P.; Qayoom, I.; Reshi, B.M.; Hasib, A.; Ahmed, N.Z. and Anwar, N. (2022). Saqmunia (*Convolvulus scammonia* L.), an important drug used in Unani system of medicine: A review, J. Drug Deliv. Therap., **12**(5):231-238.

Apak, R.; Capanoglu, E. and Shahidi, F. (Eds.) (2018). Measurement of antioxidant activity capacity. Recent Trends and Applications, doi:10.1002/9781119135388.ch5

Baliyan, S.; Mukherjee, R.; Priyadarshini, A.; Vibhuti, A.; Gupta, A.; Pandey, R.P. and Chang, C.M. (2022). Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*. Molecules (Basel, Switzerland), **27**(4):1326. https://doi.org/10.3390/molecules27041326

Balyan, P. and Ali, A. (2022). Comparative analysis of the biological activities of different extracts of *Nigella sativa* L. seeds. Ann. Phytomed., **11**:577-587.

Basha, S.K. and Sudarshanam, G. (2010). Ethnobotanical studies on medicinal plants used by sugalis of rasmalai in kurnool district, Andhra Pradesh, India. Int. J. Phytomed., **2**:349-353.

Beppu, H.; Koike, T.; Shimpo, K.; Chihara, T.; Hoshino, M.; Ida, C. and Kuzuya H (2003). Radical-scavenging effects of *Aloe arborescens* Miller on prevention of pancreatic islet B-cell destruction in rats. J. Ethnopharmacol., **89**:37-45. doi:10.1016/S0378-8741(03)00268-X.

Borins, M. (1987). Traditional medicine in India. Can. Fam. Physician., **33**:1061-1065.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., **72**:248-254. https://doi.org/10.1006/abio.1976.9999.

Das, S.; Bala, A.; Bhowmik, M. and Ghosh, L.K. (2012). Attenuation of reactive nitrogen species by different flavonoids enriched fractions of Schimawallichii. Asian Pac. J. Trop. Med., **2**(2):S632-S636. https://doi.org/10.1016/S2221-1691(12)60287-1.

Gupta, S. and Ved, A. (2017). *Operculina turpethum* (Linn.) Silva Manso as a medicinal plant species: A review on bioactive components and pharmacological properties. Pharmacog. Rev., **11**(22):158-166. doi: 10.4103/phrev.phrev_6_17.

Gusakov, A.V.; Kondratyeva, E.G. and Sinitsyn, A.P. (2011). Comparison of two methods for assaying reducing sugars in the determination of carbohydrase activities. Int. J. Anal. Chem., 283658. https://doi.org/10.1155/2011/283658

Hassan Sher, H. A. J.; Abbasi, A. M.; Rahman, I. U.; Hussain, W.; Bussmann, R. W. and Paniagua-Zambrana, N. Y. (2021). *Colchicum aitchisonii* (Hook. f.) Nasir *Colchicum luteum* Baker. Doi: 10.1007/978-3-030-57408-6_63.

Khandelwal, K.R. (2006), Practical Pharmacognosy: Techniques and Experiments, Nirali Prakashan Pune, India, pp:150-156.

Khatoon, S.; Shoaib, M.; Azmat, J. and Anwar, M. (2023). Concept of Waja ál-Mafasil or Hudar (Rheumatoid Arthritis) in Unani Medicine: A review. Int. J. Herb Med., **11**(1):15-21. DOI: 10.22271/flora.2023.v11.i1a.848

Kunnumakkara, A. B.; Banik, K.; Bordoloi, D.; Harsha, C.; Sailo, B. L.; Padmavathi, G. and Roy, N.K., (2018). Googling the guggul (*Commiphora* and *Boswellia*) for prevention of chronic diseases. Front. Pharmacol., **9**:686.

Lalhminghlui, K. and Jagetia, G.C. (2018). Evaluation of the free-radical scavenging and antioxidant activities of Chilauni, Schimawallichii Korth *in vitro*. Future Sci., **4**(2):FSO272. https://doi.org/10.4155/fsoa-2017-0086

Lu, X.; Wang, J.; Al-Qadiri, H.M.; Ross, C.F.; Powers, J.R.; Tang, J. and Rasco, B.A. (2011). Determination of total phenolic content and antioxidant capacity of onion (*Allium cepa*) and shallot (*Allium oschaninii)* using infrared spectroscopy. Food Chem., **129**(2):637-644. https://doi.org/10.1016/j.foodchem.2011.04.105

Matsuura, H.N. and Fett-Neto, A.G. (2015). Plant alkaloids: Main features, toxicity, and mechanisms of action. In: Plant Toxins (eds. P. Gopalakrishnakone, C. Carlini C and R. Ligabue-Braun), Toxicology. Springer, Dordrecht, pp:1-15. https://doi.org/10.1007/978-94-007-6728-7_2-1.

Ozgen, M.; Schreerens, J.C.; Reese, R.N. and Miller, R.A. (2010). Total phenolic, anthocyanidin contents and antioxidant capacity of selected elderberry (*Sambucus canadensis* L.) accession. Pharmacog. Mag., **6**:198-203.

Sánchez, G; Senizza, B., Zengin, G; Okur, M.A.; Montesano, D.; Yildiztugay, E.; Lobine, D.; Mahomoodally, M.F. and Lucini, L. (2019). Chemical profiling and biological properties of extracts from different parts of *Colchicum szovitsii* Subsp. Szovitsii. Antioxidants (Basel), **11**:8(12):632. doi: 10.3390/an,tiox8120632.

Sánchez, M.; González-Burgos, E.; Iglesias, I. and Gómez-Serranillos M.P. (2020). Pharmacological update properties of *Aloe vera* and its major active constituents. Molecules, **25**(6):1324. doi: 10.3390/molecules 25061324.

Shaikh, J.R. and Patil, M.K (2020). Qualitative tests for preliminary phytochemical screening: An overview. Int. J. Chem. Stud., **8**:603-608. https://doi.org/10.22271/chemi.2020.v8.i2i.8834

Shraim, A.M.; Ahmed, T.A.; Rahman, M.Z. and Hijji, Y.M. (2021). Determination of total flavonoid content by aluminium chloride assay: A critical evaluation, LWT, 150:111932. https://doi.org/10.1016/j.lwt.2021.111932

Tarannum, A.; Sultana, A. and Ur Rahman, K. (2016). Clinical efficacy of certain Unani herbs in knee osteoarthritis: A pretest and post-test evaluation study. Anc. Sci. Life., **35**(4):227-31. doi: 10.4103/0257-7941.188185.

Tichy, J. and Novak, J. (1998). Extraction, assay, and analysis of antimicrobials from plants with activity against dental pathogens (*Streptococcus* sp.) J. Altern. Complement. Med., **4**(1):39-45.

Tungmunnithum, D.; Thongboonyou, A.; Pholboon, A. and Yangsabai A (2018). Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. Medicines., 5:93. doi: 10.3390/medicines5030093.

Youssef, F.S.; Eid, S.Y.; Alshammari, E.; Ashour, M.L.; Wink, M. and El-Readi, MZ (2020). *Chrysanthemum indicum* and *Chrysanthemum morifolium*: Chemical composition of their essential oils and their potential use as natural preservatives with antimicrobial and antioxidant activities. Foods, 14::1460. doi: 10.3390/foods9101460.

Zhu, Z.; Li, S.; He, J.; Thirumdas, R.; Montesano, D. and Barba, F.J. (2018). Enzyme-assisted extraction of polyphenol from edible lotus (Nelumbo nucifera) rhizome knot: Ultra-filtration performance and HPLC-MS2 profile. Food Res. Int., **111**:291-298. doi: 10.1016/j.foodres.2018.05.047.

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