RESEARCH ARTICLE

Evaluation of Antioxidant Activity and FTIR Spectroscopic Analysis of Bark Extracts of *Psydrax dicoccos* Gaertn.

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Abstract

Psydrax dicoccos Gaertn., a vulnerable plant according to the IUCN Red List, holds diverse cultural and practical significance in India. This study explores the antioxidant potential of its bark, rich in phytoconstituents like saponins, flavonoids, and alkaloids. Aqueous and 70% ethanolic extracts were analyzed for total polyphenol and flavonoid content, demonstrating 70.77 ± 1.92 and 88.29 ± 2.18 mg catechol equivalent per gram, and 212.85 ± 2.61 and 745.53 ± 3.93 mg quercetin equivalent per gram, respectively. Antioxidant assays, including DPPH and ABTS, revealed the bark's significant scavenging activity, with IC_{50} values of 73.68 and 56.06 µg/mL for the aqueous extract, and 35.65 µg/mL and 16.6 µg/mL for the ethanolic extract. The reducing power assay showcased a notable reducing capacity, while the CUPRAC assay demonstrated antioxidant potential, equivalent to 127.9 ± 5.76 and 573.4 ± 5.09 mg Ascorbic Acid equivalent per gram for the aqueous and ethanolic extracts, respectively. The phosphomolybdenum method indicated antioxidant capacity, with values of 223.02 ± 1.87 and 188.40 ± 1.88 mg Ascorbic Acid equivalent per gram for the aqueous and ethanolic extracts. FTIR analysis identified various organic compounds, including alcohols, phenols, carboxylic acids, and aromatics in the bark extracts. This comprehensive investigation underscores the therapeutic potential of *Psydrax dicoccos* bark, offering valuable insights into its antioxidant properties and chemical composition. The findings contribute to the broader understanding of medicinal plants, emphasizing their role in traditional practices and potential applications in preventing or treating oxidative stress-related ailments.

Keywords: *Psydrax dicoccos*, Antioxidant activity, Phytoconstituents, Medicinal plants, Traditional medicine.

Highlights

- *Psydrax dicoccos bark* is rich in phytoconstituents and has been traditionally used due to its multifaceted medicinal properties, including anti-inflammatory, anti-fungal and anti-diabetic effects.
- Thorough evaluation of aqueous and 70% ethanolic extracts showcases robust antioxidant capacities through DPPH, ABTS, reducing power, CUPRAC, and phosphomolybdenum assays.
- FTIR analysis reveals organic compounds, including alcohols, phenols, carboxylic acids, and more, providing valuable insights into the bark's chemical composition.
- High levels of flavonoids in the ethanolic extract highlight the potential health benefits, as flavonoids are known for their antioxidant and disease-preventive properties.
- *Psydrax dicoccos* bark extract study explores its potential as a sustainable resource for pharmaceuticals, emphasizing eco-friendly alternatives in medicinal research.

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Introduction

Oxidative stress arises from an imbalance in the generation and accumulation of reactive oxygen species (ROS) within cells and tissues, coupled with the body's capacity to counteract these reactive entities. This state not only contributes to the onset of various chronic and degenerative diseases but also expedites the aging process and leads to acute conditions like trauma and stroke (Pizzino *et al*., 2017). Numerous investigations underscore the essential role of antioxidants in maintaining human health, and preventing, and treating diseases by mitigating oxidative stress (Munteanu and Apetrei, 2021). Antioxidants are defined as any substance that significantly delays or prevents the oxidation of an oxidisable substrate when present at low concentrations (Halliwell and Gutteridge, 1995). On the basis of their mechanism antioxidants can be divided into three types: 1) Primary antioxidants-which primarily act as free radical scavengers; 2) Secondary antioxidants-which are important preventive antioxidants that work by delaying chain initiation; and 3) Tertiary antioxidants-which work to repair the biomolecules which are damaged (Daramola and Adegoke, 2011). Antioxidants can be enzymatic as well as non-enzymatic. Enzymatic antioxidants are further divided into primary enzymes, including catalase, superoxide dismutase, and glutathione peroxidase, and secondary enzymes such as glutathione reductase and glucose-6-phosphate dehydrogenase (Carocho and Ferreira, 2013).The protective role of enzymatic antioxidants against oxidative stress involves intricate interactions among various antioxidant enzymes. Superoxide, released during oxidative phosphorylation, undergoes conversion to hydrogen peroxide, which is subsequently reduced

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to water. This detoxification process relies on multiple enzymes, with superoxide dismutases transforming superoxide into hydrogen peroxide, further eliminated by various peroxidases and catalases (Lobo *et al*., 2010). Non-enzymatic antioxidants encompass a diverse range, including phenolic acids (e.g., ferulic acid), flavonoids (e.g., quercetin), vitamins and their derivatives (e.g., vitamin A, E), carotenoids (e.g., lycopene), cofactors (e.g., Coenzyme Q10), minerals (e.g., zinc, selenium), organosulfur compounds (e.g., indoles), and nitrogen non-protein compounds (e.g., uric acid) (Carocho and Ferreira, 2013).

Psydrax dicoccos Gaertn., a member of the Rubiaceae family, presents itself as a large, erect shrub or small tree with an unarmed nature. Its young shoots exhibit a distinctive four-angled structure. The leaves, elliptic-lanceolate in shape, feature an acuminate apex, a narrow base, entire margins, and a coriaceous, shining, glabrous surface. The petiole is short, and stipules are present. White, five-merous flowers are observed in axillary clusters, with glabrous and slender pedicels. The fruit type is a subglobose drupe (Almeida, 2001). As per IUCN Red list it is a vulnerable plant.

In India, the fruit pulp of *Psydrax dicoccos* Gaertn. is reported to be edible (Bhagat *et al*., 2016). The plant holds significance in traditional practices, where the bark is utilized for treating fever, applied as plaster and a decoction of the roots is employed to address diarrhoea (Tchamgoue *et al*., 2023). Among the Malayali tribals residing in the Kolli hills of Tamil Nadu, India, a leaf extract mixed with banana is orally administered to facilitate delivery (Vaidyanathan *et al*., 2013). Beyond medicinal uses; the leaf, bark and root of the plant are used as a fish poison (Chukwudulue *et al*., 2022). These diverse applications showcase the multi-faceted role of *Psydrax dicoccos* in various cultural and practical contexts.

Psydrax dicoccos is rich in phytoconstituents like saponins, flavonoids, coumarins, alkaloids, tannins and terpenes etc. which have anti-inflammatory, anti-fungal, anti-diabetic, anti-diarrheal, arthritic, febrifuge, hepatoprotective and nephroprotective properties (Mahalakshmi, 2021). Vuyyuri (2014) investigated the ethanolic extract of the whole plant of *Psydrax dicoccos*, revealing its anti-inflammatory activity in Wistar albino rats across different models, including carrageenan-induced paw oedema, formalin-induced paw oedema, fresh egg white-induced paw oedema, and cotton pellet-induced granuloma model. The results suggested the extract's potential as an alternative to NSAIDs like Diclofenac. Furthermore, various solvent extracts were evaluated for antimicrobial activity, with a methanol extract displaying the highest efficacy against a range of bacteria and fungi (Umaiyambigai *et al*., 2015).

Materials And Methods

Collection of Plant Material

The bark of *Psydrax dicoccos* Gaertn. was collected on September 7, 2022, from the hilltop of Mt. Barry, Matheran, Maharashtra. The plant specimen was authenticated by comparing it with voucher specimen no. 5861 of H.Santapau S.J. at the Blatter herbarium, St. Xavier's College, Mumbai. The bark material collected was kept in a hot air oven at 40°C for drying. After complete drying, it was powdered with the help of a mixer and the powder was stored in an airtight container.

Chemicals

DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), L – Ascorbic Acid 99% Extra pure, Quercetin, Catechol, Sodium carbonate, Folin-Ciocalteu reagent, Sodium nitrite, Aluminium chloride, Sodium hydroxide, Sulphuric acid, Ammonium molybdate, Sodium phosphate, Trichloroacetic acid, Potassium ferricyanide, Ferric chloride, Potassium persulfate, Neocuproine, Ammonium acetate, Cupric chloride. All the chemicals used in the analysis were of analytical grade.

Extract Preparation

The dried powder of Bark (3 gm) was soaked in 30 mL of Distilled water and 70% Ethanol respectively. It was kept on an orbital shaker for 24 hours. Later on, the extract was filtered using Whatman No. 1 filter paper. The filtrate was evaporated to complete dryness using a water bath to get the dry extracts.

Quantitative Analysis of Polyphenols and Flavonoids

Total Polyphenol Content

Total Polyphenol Content was estimated using the method of (Dibacto, R.E. *et al*., 2021). A volume of 0.5 mL of extract was added with 2.5 mL of 0.2 N Folin-Ciocalteu reagent and later 2 mL of 7.5% Sodium carbonate was added. The tubes were nicely vortexed. It was kept for incubation at room temperature for half an hour. Later the absorbance was measured at 765 nm against blank using ELISA Plate Reader (SpectraMax iD3). Catechol was used as a standard.

Total Flavonoid Content

Total flavonoid content was estimated using the method described by (Baba and Malik, 2015) with minor modifications. 1 mL of extract was added with 4 mL of distilled water. Then 0.3 mL of 5% Sodium nitrite was added. The tubes were kept for 5 minutes. Then 0.3 mL of 10% aluminium chloride was added which was followed by 2 mL of Sodium hydroxide (1 M). The volume of tubes was made up to 10 mL with distilled water. Later the absorbance was measured at 510 nm against blank using ELISA Plate Reader (SpectraMax iD3). Quercetin was used as a standard.

Antioxidant Analysis

DPPH Assay

The DPPH assay was performed using a microplate method of (Prieto, 2012). The DPPH solution was prepared in methanol. A volume of 100 µL of extract and standard with varying concentrations was added with 100 µL of 0.2 mM DPPH solution in each well. After the lid was put back , the plate was covered with aluminium foil and incubated at room temperature in the dark for 30 minutes. Later the absorbance was measured at 517 nm using the ELISA Plate Reader (SpectraMax iD3). In control 100 µL of distilled water and 70% Ethanol was used instead of sample. Ascorbic acid was used as a standard. The percent inhibition is calculated using the following formula.

$$\% \text{ Inhibition} = \frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} \times 100$$

ABTS Assay

The ABTS assay was performed using the method of (Lee *et al*., 2015). To prepare ABTS reagent First 7 mM ABTS solution and 140 mM Potassium persulfate solution was prepared in water. Later 88 µL of 140 mM Potassium persulfate solution was mixed with 5 mL of 7 mM ABTS solution. In order to allow free radicals to generate it was kept at room temperature in the dark for 16 hours. Then it was diluted using water (1 : 44, v/v). It was performed using a microplate method. A volume of 100 µL of extract and standard with varying concentrations was added with 100 µL of ABTS reagent in each well. After the lid was put back, the plate was covered with aluminum foil and incubated at room temperature in the dark for 6 minutes. Later the absorbance was measured at 734 nm using the ELISA Plate Reader (SpectraMax iD3). In control 100 µL of distilled water and 70% Ethanol was used instead of sample. Ascorbic acid was used as a standard. The percent inhibition is calculated using the following formula.

$$\% \text{ Inhibition} = \frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} \times 100$$

Reducing Power

Reducing power was performed using the method of Oyaizu described by (Bhalodia *et al*., 2013). A volume of 1mL of extract and standard with varying concentrations was mixed with 2.5 mL of 0.2 M Phosphate buffer with pH 6.6. Later 2.5 mL of 1% Potassium ferricyanide was added. The tubes were vortexed nicely. The mixture was incubated at 50 °C for 20 minutes. The tubes were allowed to cool down. Then 2.5 mL of 10% Trichloroacetic acid was added. Mixture was centrifuged at 3000 rpm for 10 minutes. After centrifugation , 2.5 mL from the upper layer of supernatant is mixed with 2.5 mL of distilled water. At last, add 0.5 mL of 0.1% Ferric chloride. . Later the absorbance was measured at 700 nm against blank using ELISA Plate Reader (SpectraMax iD3). Ascorbic acid was used as a standard.

CUPRAC Assay

CUPRAC Assay was performed using the method of (Özyürek *et al*., 2011). In a test tube 1mL of each of 7.5 mM Neocuproine, 1 M Ammonium acetate buffer (pH 7), 10 mM Cupric chloride solutions were added. In this premixed solution , 1 mL of extract was added. The tubes were nicely vortexed. Then it was allowed to incubate at room temperature. Later the absorbance was measured at 450 nm against blank using ELISA Plate Reader (SpectraMax iD3). Ascorbic acid was used as a standard.

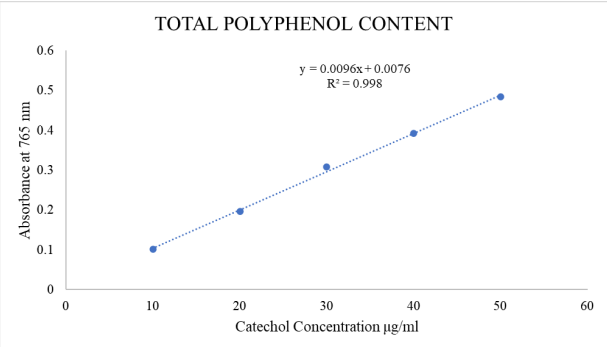

Fig. 1: Standard curve of Catechol

Phosphomolybdenum method

Total antioxidant capacity was determined using the method of Prieto described by (Zahin *et al*., 2010). In an Eppendorf tube 100 µL of extract was added. To the extract 1 mL of Reagent solution (28 mM Sodium Phosphate, 4 mM Ammonium molybdate and 0.6 M Sulphuric acid) was added. The tubes were closed properly and kept in a water bath at 95° C for 90 minutes. Then the tubes were allowed to cool down. Later the absorbance was measured at 695 nm against blank using ELISA Plate Reader (SpectraMax iD3). Ascorbic acid was used as a standard.

FTIR Analysis

FTIR (Fourier Transform Infrared) spectroscopy has made an impressive role in the field of medicinal plant analysis (Nair *et al*., 2013). It is an extensively used method to identify the chemical compounds and elucidate their structures. Due to the diagnostic characters and vast applicability to the samples, FTIR has played a crucial role in pharmaceutical research in recent years (Baseri and Baker, 2011). FT-IR (Fourier Transform Infrared Spectroscopy) analysis provides quantitative and qualitative spectral information that is essentially a molecular fingerprint for organic, inorganic and polymeric compounds. FTIR identifies chemical bonds in a molecule by producing an infrared absorption spectrum. The FTIR spectrum of the test sample can be compared with the libraries of spectra that have been catalogued for known compounds. FTIR peaks are relatively narrow and, in many cases, they can be associated with the vibration of a particular chemical bond (or a single functional group) in the molecule. The dry extract powder of the sampled plants was scanned at room temperature (25 ± 2°C) at spectral range of 4000 - 450 cm^{-1} using Spectrum Two FTIR by PerkinElmer. For the noise reduction of each spectrum, the spectral resolution was set to 4.0 cm^{-1}. The spectrum of each sample was recorded with the software NIOS2 Main. Background spectra collected under identical conditions were subtracted from the sample spectrum. Interpretations of the peaks obtained in the spectrum were done by referring to standard FTIR tables for assigning corresponding functional groups (Ragavendran *et al*., 2011; Kumar and Prasad, 2011).

Statistical Analysis

All the assays were carried out in triplicates. Results of all the assays carried out were expressed as mean ± SD. Experimental data were analysed by variance analysis (One-way ANOVA). When the *p-value* < 0.05, then the difference is considered to be statistically significant.

Results

Total Polyphenol Content

Phenolic compounds are widely distributed in the plants. They are the most abundant secondary metabolites present in the plants. Due to their strong antioxidant properties and notable impacts in the prevention of oxidative stress related diseases such as cancer, plant phenolics have gained growing attention (Dai and Mumper, 2010). Total polyphenol content was determined using the Folin-Ciocalteu method. A standard curve was obtained using Catechol as shown in Fig. 1. Total

polyphenol content of aqueous and 70% ethanolic extracts of bark was 70.77 ± 1.92 and 88.29 ± 2.18 mg catechol equivalent per gram of sample, respectively.

Total Flavonoid Content

Specifically, flavonoids are a sort of secondary metabolite of plants with a polyphenolic structure that are prevalent in fruits, vegetables, and some drinks. They constitute a significant class of natural substances. They have many beneficial biochemical and antioxidant effects associated with diseases such as atherosclerosis, cancer, Alzheimer's disease etc. Naturally, flavonoids can be extracted from the plant. Various parts of the plant contain flavonoids (Panche *et al.*, 2016). Total Flavonoid content was determined using the aluminium chloride method. A standard curve was obtained using quercetin as shown in Fig. 2. Total flavonoid content of aqueous and 70% ethanolic extracts of bark was 212.85 ± 2.61 and 745.53 ± 3.93 mg quercetin equivalent per gram of sample, respectively.

DPPH Assay

DPPH assay is used to measure scavenging capacity of antioxidants against free radicals. DPPH is a free stable radical which has a deep purple colour. When an antioxidant donates an electron or hydrogen atom the DPPH radical gets neutralized and the DPPH gets reduced to DPPH – H.

DPPH radical absorbs strongly at 517 nm, giving rise to a deep purple colour. Nevertheless, when an odd electron pairs up with another electron, the initial colour gradually decolorizes into pale yellow (Bibi Sadeer *et al.*, 2020). Percent Inhibition of Aqueous and 70% Ethanolic extracts of Bark were compared with Ascorbic Acid with a fixed concentration range, as shown in Fig. 3. IC_{50} value is the amount of concentration required to scavenge 50% radicals. The IC_{50} values of Aqueous and 70% Ethanolic extract of bark were 73.68 and 35.65 µg/mL respectively. IC_{50} value of Ascorbic acid was 12.86 µg/mL. Lower the IC_{50} value better is the antioxidant activity.

ABTS Assay

ABTS assay measured the scavenging capacity of antioxidants against free radicals. When the $ABTS^{\bullet+}$ radical, which is in its unstable form, accepts an electron from the antioxidant, it exhibits a blue-green colour. The colour fades into a pale blue, indicating stable form of ABTS regeneration (Bibi Sadeer *et al.*, 2020). Percent Inhibition of Aqueous and 70% Ethanolic extracts of Bark were compared with Ascorbic Acid with a fixed concentration range, as shown in Fig. 4. IC_{50} value is the amount of concentration required to scavenge 50% radicals. The IC_{50} values of aqueous and 70% Ethanolic extract of bark were 56.06 and 16.6 µg/mL, respectively. IC_{50} value of ascorbic acid was 4.38 µg/mL. Lower the IC_{50} value better is the antioxidant activity.

Reducing Power

The reducing power method is based on the principle that substances possessing a reduction potential undergo a reaction

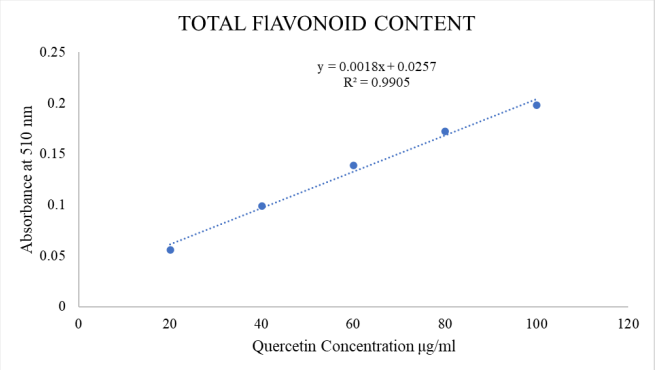

Fig. 2: Standard curve of Quercetin

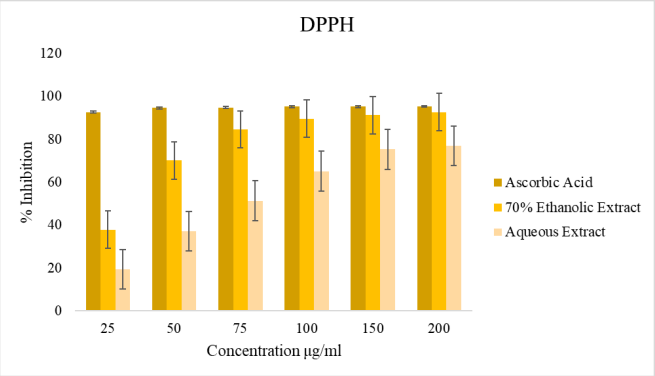

Fig. 3: Radical scavenging activities of aqueous extract, 70% ethanolic extract in comparison with standard ascorbic acid

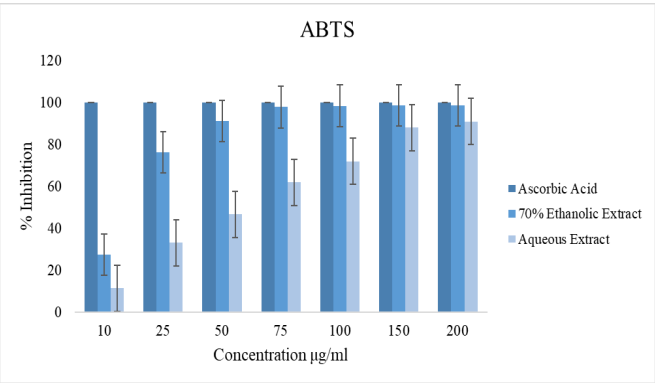

Fig. 4: Radical scavenging activities of aqueous extract, 70% ethanolic extract in comparison with standard ascorbic acid.

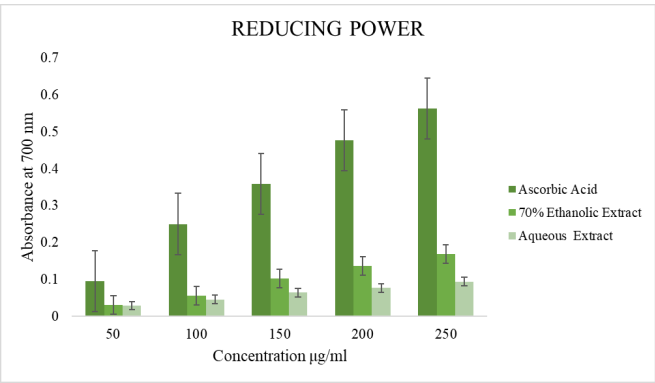

Fig. 5: Reducing power activities of aqueous extract, 70% ethanolic extract in comparison with standard ascorbic acid

with potassium ferricyanide (Fe^{3+}) to produce potassium ferrocyanide (Fe^{2+}). Subsequently, this reduced form Fe^{2+} reacts with ferric chloride to produce a ferric–ferrous complex (bluish green color) with an absorption maximum at 700 nm (Bhalodia *et al.*, 2013). Absorbance values of Aqueous and 70% Ethanolic extracts of Bark were compared with Ascorbic Acid with a fixed concentration range, as shown in Fig. 5. Higher the absorbance, higher the reducing capacity.

CUPRAC Assay

Indeed, this technique has been demonstrated to be efficacious for a multitude of polyphenols, including but not limited to phenolic acids, hydroxycinnamic acids, flavonoids, carotenoids, and anthocyanins, as well as thiols, synthetic AOs, and vitamins C and E. The chromogen employed in this experiment is a bis(neocuproine) copper (II) cation, namely [Cu $(Nc)_2^{2+}$]. Upon its reduction by an antioxidant, the light blue chromophore undergoes a reduction to form an orange-yellow bis(neocuproine) copper (I) chelate [Cu $(Nc)_2^{+}$] that can be read at 450 nm (Bibi Sadeer *et al.*, 2020). A standard curve was obtained using Ascorbic acid as shown in Fig. 6. CUPRAC capacity of Aqueous and 70% Ethanolic extracts of bark was 127.9 ± 5.76 and 573.4 ± 5.09 mg Ascorbic Acid equivalent per gram of sample, respectively.

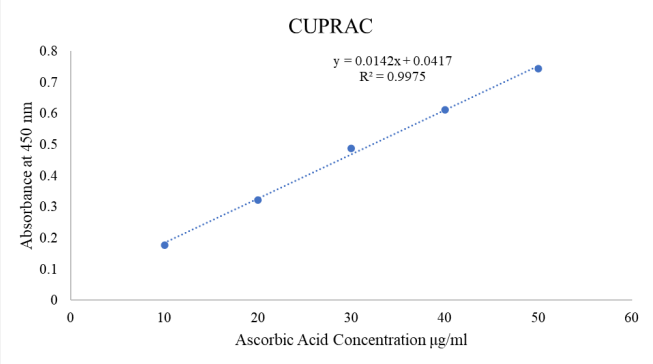

Fig. 6: Standard curve of Ascorbic Acid

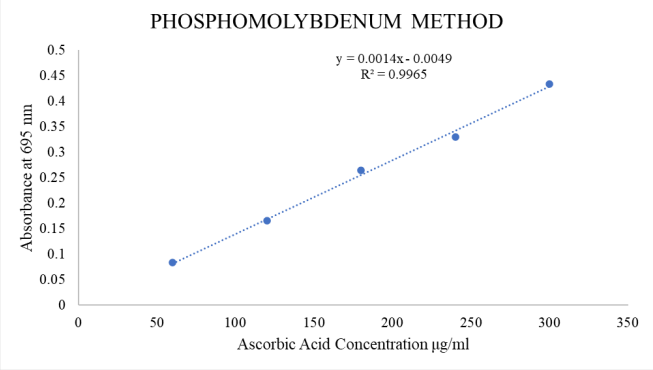

Fig. 7: Standard curve of Ascorbic Acid

Phosphomolybdenum Method

Phosphomolybdenum method is used to find out the antioxidant capacity of extract through the formation of phosphomolybdenum complex. It is based on the reduction of Mo (VI) to Mo (V) by using a sample analyte that forms green phosphate Mo (V) at acidic conditions (Pavagadhi *et al.*, 2012). A standard curve was obtained using Ascorbic acid as shown in Fig. 7. Total antioxidant capacity of aqueous and 70% ethanolic extracts of bark was 223.02 ± 1.87 and 188.40 ± 1.88 mg ascorbic Acid equivalent per gram of sample, respectively.

FTIR Analysis

The aqueous extract of bark powder of *Psydrax dicoccos* exhibited seven peaks with different characteristic bands in the FT-IR analysis of the sample as represented in Table 1 and Fig. 8. Whereas ethanolic extract of bark powder of *Psydrax dicoccos* exhibited nine peaks with different characteristic bands in the FT-IR analysis of the sample as represented in Table 2 and Fig. 9. For the former extract highest peak values were at 3310.62 cm-1 showed the presence of O-H group and lowest peak at 1021.03 cm-1 showing presence of C-O group. For the later one, highest peak was observed at 3342.91 cm-1 showing presence of O-H group and lowest peak observed at 1035.68 cm-1 showing presence of S=O group. Overall results of aforementioned figures confirmed the presence of various compounds belonging to organic families such as alcohols, phenols, carboxylic acid, carbonyls, nitro compounds, aromatics, amines, alkynes and alkyl halides in the bark extract.

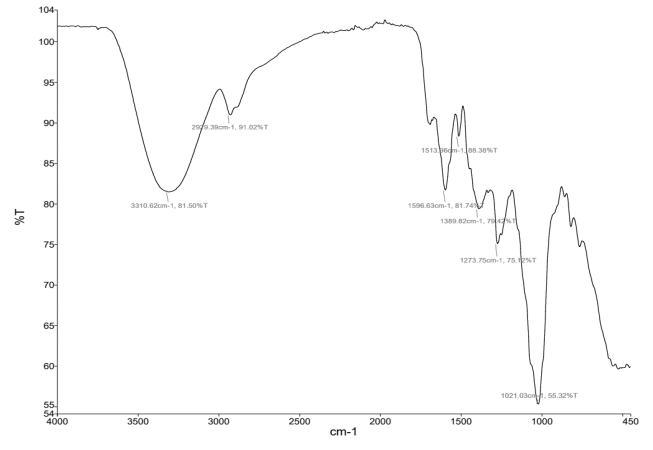

Fig. 8: FTIR analysis of Aqueous extract of *Psydrax dicoccos* Gaertn.

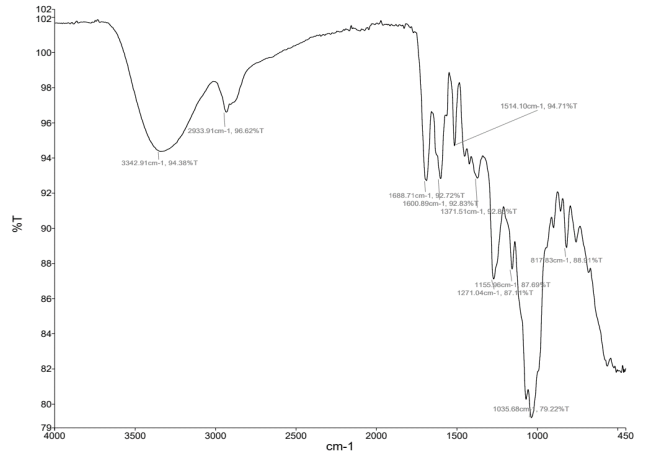

Fig. 9: FTIR analysis of 70% Ethanolic extract of *Psydrax dicoccos* Gaertn.

Table 1 : FTIR Peak values of Aqueous extract of *Psydrax dicoccos* Gaertn.

S. No.	*Peak Values*	*Functional group*
1	3310.62	O-H
2	2929.39	C-H
3	1596.63	N-H
4	1513.96	N-O
5	1389.82	O-H
6	1273.75	C-O
7	1021.03	C-O

Table 2 : FTIR Peak values of 70% Ethanolic extract of *Psydrax dicoccos* Gaertn.

S. No.	*Peak Values*	*Functional group*
1	3342.91	O-H
2	2933.91	C-H
3	1688.71	C=O
4	1600.89	C=C
5	1514.10	N-O
6	1371.51	O-H
7	1271.04	C-O
8	1155.96	S=O
9	1035.68	S=O

Discussion

The results showed that the bark's 70% ethanolic and aqueous extracts both have strong antioxidant activity. When compared to the aqueous extract, the ethanolic extract had a higher content of polyphenols and flavonoids, which was consistent with its improved antioxidant potential. These findings are consistent with earlier research showing the relationship between antioxidant activity, flavonoid concentration, and polyphenols. The ethanolic extract showed lower IC_{50} values than the aqueous extract and comparable activity to the reference standard Ascorbic acid, in the DPPH and ABTS assays, demonstrating the extract's capacity to scavenge free radicals. Both extracts had a considerable reducing ability, as demonstrated by the reducing power assay and CUPRAC assay, with the ethanolic extract showing more antioxidant potential. These findings are in line with the existence of several phytochemicals, such as flavonoids and phenolic compounds, that have antioxidant qualities. By calculating the extract's total antioxidant capacity, the phosphomolybdenum technique provided additional evidence of their antioxidant activity. Both the ethanolic and aqueous extracts shown strong antioxidant activity in the results, with the ethanolic extract displaying somewhat lower values, most likely as a result of differences in composition or extraction efficiency. The chemical makeup of the bark extracts was revealed by the FTIR analysis, which also showed the existence of several functional groups linked to antioxidant chemicals. The presence of organic components such aromatics, phenols, carboxylic acids, and alcohols is further evidence of the extract's high antioxidant content.

Conclusion

The study aimed to investigate the potential antioxidant properties of the bark of *Psydrax dicoccos* Gaertn. This plant is known for its rich phytochemical composition and traditional medicinal uses. The study utilized a range of tests to evaluate the antioxidant activity of the bark and characterized its phytochemical composition using FTIR analysis. The results showed that both the 70% ethanolic and aqueous extracts of the bark contain high concentrations of flavonoids and polyphenols, with greater values observed in the ethanolic extract. The extracts exhibited strong antioxidant activity in various assays including DPPH, ABTS, reducing power, CUPRAC, and phosphomolybdenum, with the ethanolic extract consistently outperforming the aqueous extract. These findings suggest that *Psydrax dicoccos* bark could serve as a potential natural source of antioxidants. Moreover, the FTIR analysis revealed the presence of a variety of organic chemicals including alkynes, alkyl halides, aromatics, amines, carbonyls, carboxylic acids, and alcohols. This analysis provides a more comprehensive understanding of the bioactive substances present in the bark of *Psydrax dicoccos*. Given its antioxidant-rich profile and traditional medicinal uses, this plant has the potential for further research and development in the pharmaceutical and nutraceutical industries. However, further research is required to identify the specific bioactive components responsible for the observed antioxidant activity and to assess the plant's medicinal potential for different ailments.

In summary, this study highlights the strong antioxidant properties of *Psydrax dicoccos* bark and opens up opportunities for future research into its pharmacological and therapeutic qualities. This plant offers the possibility of discovering new natural medicines and pharmaceutical interventions

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Author Contribution

Shubham Patkar designed and performed the experiments, analysed the results, and produced the paper. Saif Khan edited and critically reviewed the manuscript before approving the final version for publication.

Conflict Of Interest

None.

References

Almeida, M. R. (2001). Flora of Maharashtra—Vol. 3A. *Blatter Herbarium, St. Xavier's College, Mumbai*, 116.

Baba, S.A. and Malik, S.A. (2015) 'Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *arisaema jacquemontii* blume', *Journal of Taibah University for Science*, 9(4), pp. 449–454. https://doi.org/10.1016/j.jtusci.2014.11.001

Baseri, M. K., & Baker, S. (2011). Identification of cellular components of medicinal plants using FTIR. *Rom. J. Biophys*, *21*, 277-284.

Bhalodia, N., Nariya, P., Shukla, V., & Acharya, R. (2013). In vitro antioxidant activity of hydro alcoholic extract from the fruit pulp of Cassia fistula Linn. *AYU (An International Quarterly Journal of Research in Ayurveda)*, *34*(2), 209. https://doi.org/10.4103/0974-8520.119684

Bhagat, R. B., Chambhare, M., Mate, S., Dudhale, A., & Zaware, B. N. (2016). Prospective wild edible fruit plants from part of northern Western Ghats (NWG), Mulshi (MS), India. *Journal of Medicinal Plants*, *4*(1), 15-19.

Bibi Sadeer, N., Montesano, D., Albrizio, S., Zengin, G., & Mahomoodally, M. F. (2020). The versatility of antioxidant assays in food science and safety—chemistry, applications, strengths, and limitations. *Antioxidants*, *9*(8), 709. https://doi.org/10.3390/antiox9080709

Carocho, M., & Ferreira, I. C. F. R. (2013). A review on Antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food and Chemical Toxicology*, *51*, 15–25. https://doi.org/10.1016/j.fct.2012.09.021

Chukwudulue, U. M., Attah, A. F., & Okoye, F. B. (2022). Linking phytochemistry to traditional uses and pharmacology of an underexplored genus – Psydrax: A Review. *Phytochemistry Reviews*, *21*(5), 1577–1604. https://doi.org/10.1007/s11101-021-09798-6

Dai, J. and Mumper, R.J. (2010) 'Plant Phenolics: Extraction, analysis and their antioxidant and anticancer properties', *Molecules*, 15(10), pp. 7313–7352. https://doi.org/10.3390/molecules15107313

Daramola, B., & Adegoke, G. O. (2011). Bitter Kola (Garcinia Kola) seeds and health management potential. *Nuts and Seeds in Health and Disease Prevention*, 213–220. https://doi.org/10.1016/b978-0-12-375688-6.10025-8

Dibacto, R.E. *et al.* (2021) 'Total polyphenol and flavonoid content and antioxidant capacity of some varieties of Persea americana peels consumed in Cameroon', *The Scientific World Journal*, 2021, pp. 1–11. https://doi.org/10.1155/2021/8882594

Halliwell, B., & Gutteridge, J. M. C. (1995). The definition and measurement of antioxidants in Biological Systems. *Free Radical Biology and Medicine*, *18*(1), 125–126. https://doi.org/10.1016/0891-5849(95)91457-3

Kumar, J. K., & Prasad, A. D. (2011). Identification and comparison of biomolecules in medicinal plants of Tephrosia tinctoria and Atylosia albicans by using FTIR. *Romanian Journal of Biophysics*, *21*(1), 63-71.

Lee, K. J., Oh, Y. C., Cho, W. K., & Ma, J. Y. (2015). Antioxidant and anti-inflammatory activity determination of one hundred kinds of pure chemical compounds using offline and online screening HPLC assay. *Evidence-Based Complementary and Alternative Medicine*, *2015*, 1–13. https://doi.org/10.1155/2015/165457

Lobo, V., Patil, A., Phatak, A., & Chandra, N. (2010). Free radicals, antioxidants and Functional Foods: Impact on human health. *Pharmacognosy Reviews*, *4*(8), 118. https://doi.org/10.4103/0973-7847.70902

Mahalakshmi, P. S. (2021). In silico molecular docking analysis of selected phytoconstituents from Psydrax dicoccos (Gaertn.) against Parkinson's disease. International Journal of Green Pharmacy (IJGP), 15(3).

Munteanu, I. G., & Apetrei, C. (2021). Analytical methods used in determining antioxidant activity: A review. *International Journal of Molecular Sciences*, *22*(7), 3380. https://doi.org/10.3390/ijms22073380

Nair, L. D., Sar, S. K., Arora, A., & Mahapatra, D. (2013). Fourier transform infrared spectroscopy analysis of few medicinal plants of Chhattisgarh, India. *J. Adv. Pharm. Edu. Res*, *3*, 196-200.

Özyürek, M., Güçlü, K., Tütem, E., Başkan, K. S., Erçağ, E., Esin Çelik, S., Baki, S., Yıldız, L., Karaman, Ş., & Apak, R. (2011). A comprehensive review of CUPRAC methodology. *Analytical Methods*, *3*(11), 2439. https://doi.org/10.1039/c1ay05320e

Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: An overview. *Journal of Nutritional Science*, *5*. https://doi.org/10.1017/jns.2016.41

Pavagadhi, S., Joseph, G. S., & Jena, B. S. (2012). Antioxidant Principles in *Peltophorum Ferrugineum* Flower Extracts. *International Journal of Food Properties*, *15*(3), 549–557. https://doi.org/10.1080/10942912.2010.492544

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D., & Bitto, A. (2017). Oxidative stress: Harms and benefits for human health. *Oxidative Medicine and Cellular Longevity*, *2017*, 1–13. https://doi.org/10.1155/2017/8416763

Prieto, J.M. (2012) Procedure: Preparation of DPPH radical, and antioxidant scavenging assay. *DPPH Microplate Protocol*, 7–9.

Ragavendran, P., Sophia, D., Arul Raj, C. & Gopalakrishnan, V.K. (2011) Functional group of various extracts of Aerva lanata (L.) by FTIR spectrum. *Pharmacologyonline*, 1, 358–364.

Tchamgoue, J., Tchokokam, Y. R., Ngouonpe, A. W., Ngandjui, Y. A., Tiani, G. L., Msagati, T. A. M., Ngadjui, B. T., Green, I. R., & Kouam, S. F. (2023). The genus canthium: A comprehensive summary on its traditional use, phytochemistry, and pharmacological activities. *Fitoterapia*, *172*, 105754. https://doi.org/10.1016/j.fitote.2023.105754

Umaiyambigai, D., Saravanakumar, K. & Raj, G.A. (2015) Phytochemical profiles, antibacterial and antifungal activity of leaves from the Psydrax dicoccos (Gaertn). *Indo–Asian Journal of Multidisciplinary Research (IAJMR)*, 2, 443–452.

Vaidyanathan, D., Senthilkumar, M.S. & Basha, M.G. (2013) Studies on ethnomedicinal plants used by Malayali tribals in Kolli Hills of Eastern Ghats, Tamil Nadu, India. *Asian Journal of Plant Science and Research*, 3, 29–45.

Vuyyuri, B. (2014) Anti-inflammatory activity of ethanolic extract of Canthium dicoccum. *International Journal of Pharmaceutical and Phytopharmacological Research*, 3.

Zahin, M., Ahmad, I. and Aqil, F. (2010) 'Antioxidant and antimutagenic activity of Carum Copticum Fruit extracts', *Toxicology in Vitro*, 24(4), pp. 1243–1249. https://doi.org/10.1016/j.tiv.2010.02.004