Pharmacophore

ISSN-2229-5402

Journal home page: http://www.pharmacophorejournal.com

INVESTIGATION OF TOTAL PHENOLIC, TANNINS, FLAVONOID CONTENTS, AND ANTIOXIDANT ACTIVITY OF PISONIA ALBA

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ARTICLE INFO

Received:
14 Aug 2021
Received in revised form:
04 Dec 2021
Accepted:
09 Dec 2021
Available online:
28 Dec 2021

Keywords: Pisonia alba, Bioactive compounds, Antioxidant, Herbs

ABSTRACT

Nyctaginaceae, the Four O'Clock Family, consists of around 33 genera and 290 species and it is well recognized for its ornamental and medicinal values. Pisonia alba span is one such medicinal plant of the Nyctaginaceae family with excessive medicinal practicable and is freely reachable in India. This study was intended to determine the major class of phytochemicals evaluate the bioactivities of the crude extracts and finally identify the drug lead compounds present in *Pisonia alba*. The analyzed phytochemical of diverse extracts of *P. alba* leaf showed the presence of tannins, phenols, flavonoids, quinones, coumarins, carbohydrates, and glycosides**.** The extracts of the plants exhibited significant antioxidant activity which has potential application to reduce oxidative stress with consequent health benefits. All extracts showed a concentration-dependent increase in the antioxidant activity the findings suggest that Pisonia alba should be a conceivable supply of herbal antioxidants that should have outstanding significance as a therapeutic agent. So it is encouraged as a plant of phytopharmaceutical importance.

To Cite This Article: Sarvananda L, Premarathna A D. Investigation of Total Phenolic, Tannins, Flavonoid Contents, and Antioxidant Activity of Pisonia Alba. Pharmacophore. 2021;12(6):43-9. https://doi.org/10.51847/gQlSFWlOGP

Introduction

Nature has developed in a very remarkable way to supply more than a few healing molecules in the form of natural products. These healing Molecules can be derived from microbes to be more complicated than flora and animals. The use of medicinal flora for the cure of several illnesses can be traced lower back to the beginning of humans. Plants had been the basis of a range of hooked-up traditional systems of drugs in historic instances and influenced the development of a range of modern-day medicines [1]. the plant-based pills protected and dependable Medicinal plants have proved to the high-quality repertoire of the therapeutic molecules and it consists numerous features such as they are easily accessible, less expensive and exhibit fewer side effects and fit into the instant personal want to be used as the biological sources of herbal products. Various bioactive molecules are derived from medicinal plants. Some of the molecules can be directly used as drugs and some of them provide scaffolds, prototypes, or precursors to improve drug molecules the usage of combinatorial chemical synthesis [1].

Pisonia Alba is commonly known as moonlight tree in English, Illachaikkettayilai in Tamil is widely distributed throughout India and it is an evergreen tree. The plant is lettuce and belongs to a family called Nyctaginaceae alias a four o'clock family. Some botanists believe that it is a form of *Pisonia Grandis*. The plant can be macroscopically identified by its fluorescent green color. Pisonia alba with pale green foliage leaves is not specifically distinct from the wild P. Grandis. The plant usually grows to a height of approximately 9-12 meters with its leaf measuring 10 to 12 inches. Pisonia alba Span is native to Indonesia, especially in the east and in Java. It grows well in the forest, beaches, and other open spaces such as a hedge in the yard, in the garden as an ornamental plant or grows wild and can be found 100-300 m above sea level. The plant is abundantly found in tropical regions. In Tamil Nadu, it is mainly found in Sivagangai and Ramanathapuram districts [2].

The Scientific classification of the plant is as follows

Kingdom: Plantae

Sub-Kingdom: Angiosperms

Order: Caryophyllales

Family: Nyctaginaceae

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Tribe: Pisoniae
Genus: Pisonia

Few medicinal properties have been reported to be in Pisonia alba. Leaves, stems, and roots of this species are extensively used with the aid of the tribal humans in the practice of various folk medicines. The historical use of the plant as anti-rheumatic and antifungal. Additionally, this plant is studied for its anti-fungal, anti-oxidant, anti-microbial, anti-inflammatory, anti-diabetic, diuretic, analgesic, and wound recovery properties. Further studies reveal the presence of various phytochemical constituents mainly alkaloids, phenolic compounds, and flavonoids. This will be helpful to create interest in Pisonia alba and may be useful in developing new formulations with more therapeutic and economical value [3, 4].

Materials and Methods

Plant Collection
Healthy, disease-free leaves of Pisonia alba were collected from Teynampet, Chennai. Taxonomists recognized and authenticated the plant; the clean plant material used to be then dried underneath the shade. Dried plant material was powdered using the mechanical grinder and preserved in an air-tight container.

Extraction
The plant substances (leaves) had been air-dried at room temperature (26°C) for two weeks, after which it was ground to a uniform powder. The extracts of the leaf samples have been prepared in a sequential process by way of soaking 150 g of dried powder in 450 ml of specific solvents (Petroleum ether, Ethyl acetate, and Methanol) for 48 h. The method used to be repeated. At the top of every respective extraction, the extracts have been filtered by the usage of Whatma1 filter paper. The filtrate used to be targeted under reduced pressure in a vacuum at 40°C for 25 min the use of a rotary evaporator (Super fit-ROTAVAP, India). The percentage yield of extracts was calculated.

Phytochemical Screening
The phytochemical screening of leaf extracts was once assessed through well-known techniques (Trease and Evans, 1987; Harborne, 1994). Phytochemical screening was once carried out on the leaf extracts the usage of different solvents [Hexane, Ethyl acetate, and Methanol] to become aware of the essential natural chemical groups such as carbohydrates, tannins, saponins, flavonoids, alkaloids, quinones, glycosides, phenols, terpenoids, cardiac glycosides, coumarins, steroids, phytosterols, Phlobatannins and Anthraquinones **(Figure 1 and Table 1)**.

Quantitative Analysis of Flavonoid, Tannin and Phenolic Components **(Figure 2)**

Estimation of Total Phenolic Content (TPC)
Reagents required: Folin-Ciocalteu reagent, Sodium carbonate, and Leaf extracts (1mg/ml).
Procedure: The whole phenolic content material of the extract used to be assessed following the Folin–ciocalteau technique (Slinkard & Singleton, 1977) with some modifications. Briefly, 0.1 ml of extracts (200, 600, and 1000µg/ml), 1.9 ml distilled water, and 1 ml of Folin–Ciocalteau reagent have been seeded in a tube, and then 1 ml of 100 g/l Na2CO3 was once added. The reaction combination used to be incubated at 25 °C for 2 h and the absorbance of the combination used to be read at 765 nm. The sample was examined in triplicate and a calibration curve with six statistics factors for catechol used to be obtained. The outcomes were compared to a catechol calibration curve and the whole phenolic content material of the sample was expressed as mg of catechol equivalents per gram of extract **(Table 4)**.

Amount TPC = Sample OD/Standard OD * Respective Amount of extract (1)

Estimation of Total Flavonoid Content (TFC)
Reagents required: Distilled Water, Aluminum chloride, Sodium nitrite, Sodium hydroxide, and Leaf extracts (1mg/ml).
Procedure: The complete flavonoid content material of the extract used to be decided through a colorimetric approach as described in the literature (Zhishen, Mengcheng, &Jianming, 1999). Sample (0.5 ml) used to be blended with 2 ml of distilled water and subsequently with 0.15 ml of a NaNO2 solution (15%). After 6 min, 0.15 ml of an AlCl3 solution (10%) was added and allowed to stand for 6 min, then 2 ml of NaOH solution (4%) was added to the mixture. Immediately, water was added to deliver the final volume to 5 ml and the combination was completely combined and allowed to stand for another 15 min. The absorbance of the combination was then determined at 510 nm versus prepared water blank. Results have been expressed as quercetin equivalents (mg quercetin /g dried extract) **(Table 2)**.

Amount TFC = Sample OD/Standard OD * Respective Amount of extract (2)

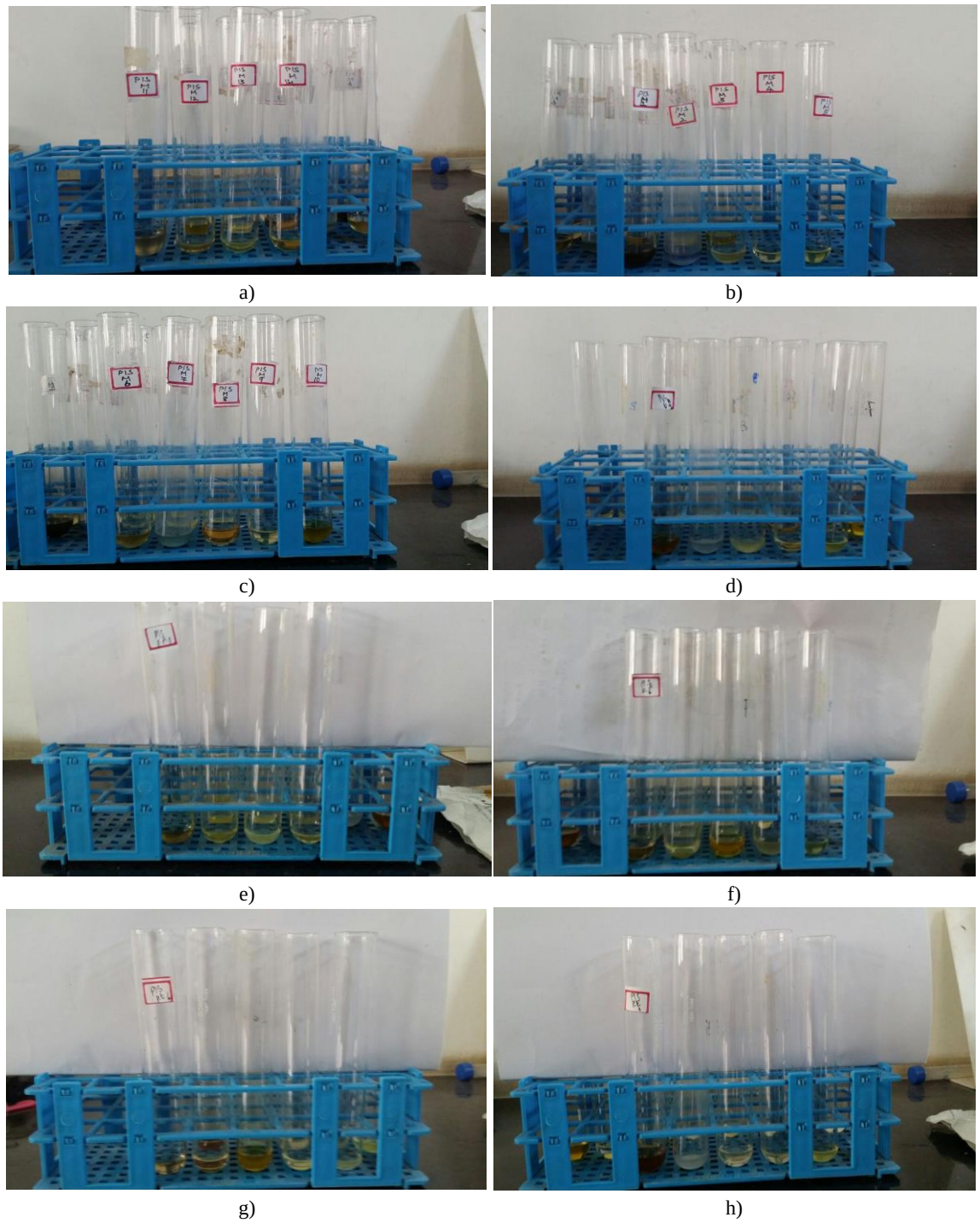
Total Tannins Content (TTC)

Reagents required: Methanol: Vanillin, Concentrated Hydrochloric Acid, and Leaf extract (1mg/ml)**.** Procedure: The evaluation of condensed tannins (proanthocyanidins) used to be carried out by the technique of Sun *et al*. (1998). To 50 µl of the accurate diluted sample, 3 ml of 4% methanol vanillin solution and 1.5 ml of concentrated hydrochloric acid were added. The combination used to be allowed to stand for 15 min, and the absorption used to be measured at 500 nm against methanol as a blank. The quantity of whole condensed tannins is expressed as mg (+)-catechin g-1. The calibration curve range used to be 0– 400 µg ml-1. All samples have been analyzed in three replications **(Table 3)**.

Amount TTC = Sample OD/Standard OD * Respective Amount of extract (3)

Results and Discussion

Phytochemical Analysis: The preliminary phytochemical analysis for the 3 extracts (Petroleum ether, Methanol, Ethyl acetate) was done according to the method described by Trease *et al.*



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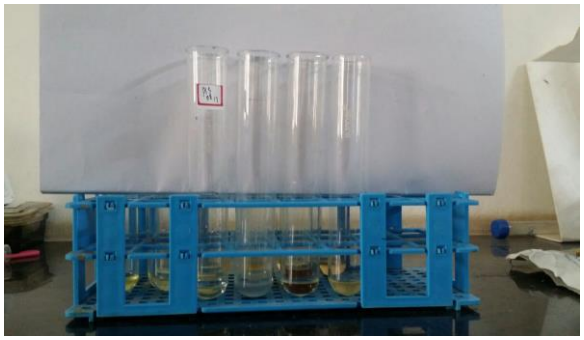

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Figure 1. Phytochemical analysis (Methanol extracts: fig a, b & c, Ethyl acetate: fig d, e & f, Petroleum ether: fig g, h & i)

Table 1. Results of the phytochemical analysis

S.No	Phytochemical Tests	Results		
		Petroleum ether Extract	Ethyl acetate Extract	Methanol Extract
1	Carbohydrates	++	++	+
2	Tannins	++	+	++
3	Alkaloids	+	+	+
4	Saponins	-	-	-
5	Flavonoid	+	+	++
6	Quinones	++	+	+
7	Glycosides	-	-	-
8	Cardiac Glycosides	-	-	-
9	Terpenoids	-	-	-
10	Phenols	++	++	+
11	Coumarins	+	+	+
12	Steroids and Phytosteroids	-	-	-
13	Phlobatannins	-	-	-
14	Anthraquinones	-	-	-

Where ++: Denotes high presence; +: Denotes presence; -: Denotes absence

Quantitative Analysis

Estimation of total Flavonoid Content

The total flavonoid content of the extracts was measured by the Aluminium chloride method in terms of Quercetin equivalent (QE). The total flavonoid content present in the samples was estimated as follows **(Table 2)**.

Table 2. Total flavonoid content estimation

	Total Flavonoid Content						
Conc (µg)	Pet ether (OD)	Methanol (OD)	Ethyl Acetate (OD)	Quercetin (OD)	Amt of flavonoid in P.E (mg equiv/g)	Amt of flavonoid in M (mg equiv/g)	Amt of Flavonoid in E.A (mg equiv/g)
200	0.1189	0.1104	0.1294	1.8859	13.72	11.70	12.60
600	0.1794	0.1209	0.1664	2.0898	47.77	34.71	51.50
1000	0.2105	0.1356	0.2084	2.5042	83.22	54.14	84.05

Estimation of Total Tannin Content

The total Tannin content of the extract was measured by Folin–Denis method in terms of Catechin equivalent (CN) was tabulated. The total tannin content of samples was estimated as follows.

Table 3. Total Tannin Estimation

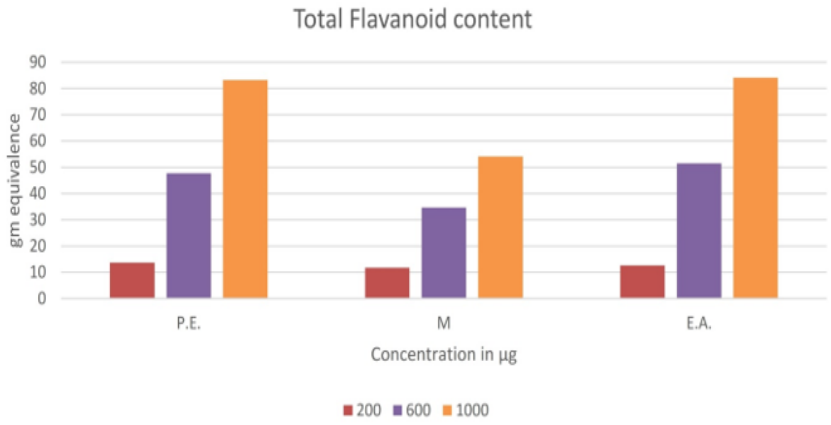
	Total Tannin Content						
Conc (µg)	Petroleum ether (OD)	Methanol (OD)	Ethyl acetate (OD)	Catechin (OD)	Amt of tannin in E.A. (mg equiv/g)	Amt of tannin in M (mg equiv/g)	Amt of tannin in P.E. (mg equiv/g)
200	0.045	0.063	0.025	0.288	17.361	43.75	31.25
600	0.126	0.193	0.103	1.462	42.27	79.20	51.70
1000	0.149	0.244	0.137	2.0846	65.72	117.04	71.47

Content of Total Phenolic Estimation (CTP)

Folin measured the total phenolic content of the extract - ciocalteau reagents in terms of Catechol equivalent (CL) are tabulated. TPC of samples was calculated as shown below.

Table 4. Total Phenolic Content

Total Phenolic Content							
Conc (µg)	**Ethyl acetate (OD)**	**Methanol (OD)**	**Petroleum ether (OD)**	**Catechol (OD)**	**Amt of phenol in P.E. (mg equiv/g)**	**Amt of phenol in M (mg equiv/g)**	**Amt of phenol in E.A. (mg equiv/g)**
200	0.123	0.1126	0.121	1.844	13.12	12.21	13.34
600	0.332	0.1966	0.2634	3.565	44.33	33.08	55.87
1000	0.4987	0.3267	0.4134	5.552	74.45	58.84	89.82



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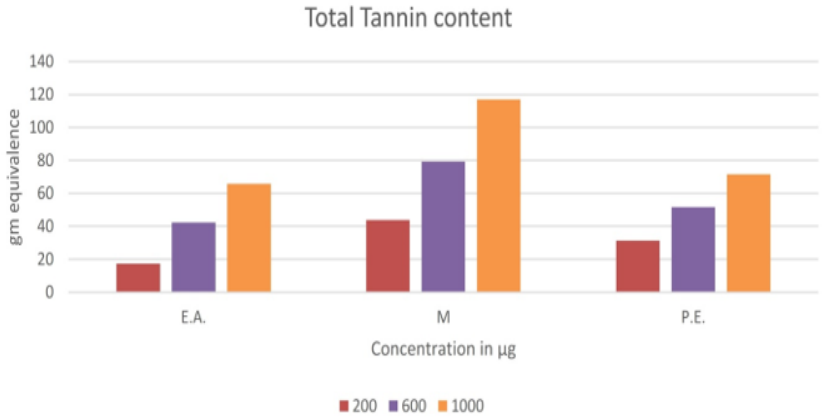

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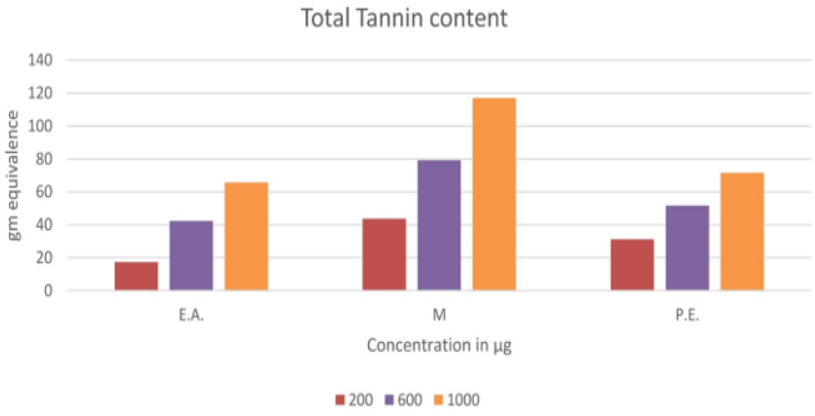

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Figure 2. Quantitative analysis of Flavonoid, Tannin and Phenolic components

Our work on *Pisonia alba* is mainly focused on understanding the Bioefficacy of the leaf extracts obtained from Petroleum ether, Ethyl acetate, and Methanol Solvents. The solvents were selected based on the increasing order of polarity. Plants embody several phytochemical constituents, many of which are recognized to be biologically energetic compounds and are

accountable for exhibiting various pharmacological activities. Some of these secondary metabolites of plant life are necessary sources of herbal antioxidants that are favored over artificial ones due to the fact of protection concerns. The secondary metabolites of bioactive are proven to decrease the threat and development of illnesses such as cardiovascular, cancer, neurodegenerative diseases, etc. by way free radicals scavenging via a range of biological mechanisms.

The outcomes of preliminary phytochemical testing demonstrated the presence of a variety of classes of bioactive chemical materials in leaf extracts of P. alba such as Carbohydrates, Tannins, Flavonoids, Quinones, phenols, and Coumarins. All of these bioactive secondary metabolites recognized in the pills have many pharmacological properties assigned to them [5]. These properties form compounds determined in the extracts of the plant recommend that they can be used in pharmaceuticals. Therefore, primarily based on the phytochemical screening results, the complete phenolic, tannin, and flavonoid contents of unique extracts were estimated.

The concentration of flavonoids in various plant extracts of *Pisonia alba* was determined using the spectrophotometric method with aluminum chloride. The highest level of flavonoid content was detected in Ethyl acetate extract of leaves 84 mg QE/gm followed by 83 mg QE/gm in Petroleum ether extract and mg QE/gm in Methanolic extract. The flavonoids are attributed to good anti-oxidant capability. The flavonoids act as natural anti-oxidants utilizing their chelating or scavenging activity. The previous work on the ethanolic extract of *Pisonia alba* reported the total flavonoid content of about 7.6 mg equivalence of quercetin/gm.

The quantitative estimation of Tannins showed good results with Methanolic extracts compared to the other two solvents. Tannins are good anti-microbial agents whose anti-microbial activity is attributed to their free radical scavenging activity. The total tannin content of the leaf samples was estimated to be 117.04 mg catechin equivalence/mg in the Methanolic extract of the plant leaves.

Folin-Ciocalteu reagent was analyzed with the total phenol contents of plant species. The total phenolic content of pomegranate flower extracts is expressed in terms of Catechol equivalent. The highest phenolic content was observed in Ethyl acetate extract of *Pisonia alba* (89 mg catechol equivalence/gm) followed by 74 mg catechol equivalence/gm in Petroleum ether extract and 58 mg catechol equivalence/gm in Methanolic extract of leaves. The completely phenolic contents in plant extracts rely on the polarity of solvent used in extraction i.e. Type of extract. Phenolic components have been recommended to play a preventive function in the development of chronic illnesses such as most cancers and coronary heart disorders [6]. Phenolic extracts additionally have been stated to retard lipid oxidation in oils and fatty ingredients [7], reduce the threat of coronary heart illnesses by using inhibiting the oxidation of low-density lipoproteins. They are also recognized to possess antibacterial, antiviral, anti-mutagenic, and anti-carcinogenic properties [8].

Based on the effects of whole phenol, tannin, and flavonoid content material in the leaves of P. alba, it can be proposed that the biological activity of this species ought to be due to the presence of flavonoids and different phenolic in it.

The current research confirmed that the presence of several bioactive compounds justifies the use of this plant for a variety of illnesses through traditional practitioners. However, the isolation of individual phytochemical components and subjecting them to the biological activity will genuinely provide fruitful outcomes. Its regular utilization in diet could arrange for health benefits to humans by protecting against oxidative stress and it should be used as an herbal source of antioxidants due to the presence of flavonoids and other phenolic compounds. Further detailed in vitro and in vivo parallel studies along with isolation of active constituents are needed to unravel novel management strategies for free radical-induced diseases.

Conclusion

These compounds studies possess necessary biological activity such as immunostimulatory, anti-inflammatory, antioxidant, anticancer, antimicrobial, thyroid inhibitory effect, lipid inhibitors, antiperoxidative and hypoglycemic effect. They indicate that the leaf extract was high in phenolic, tannins, and flavonoids compounds. The findings recommend that Pisonia Alba should be a possible source of herbal antioxidants that may want to have terrific significance as a therapeutic agent. Therefore, it is encouraged as a plant of phytopharmaceutical significance. This study can form the basis for biological characterization to the importance of the compounds acknowledged and create many bioactive ingredients to treat several diseases.

Acknowledgments: None

Conflict of interest: None

Financial support: None

Ethics statement: None

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