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ESTIMATION OF TOTAL FLAVONOIDS AND TOTAL PHENOLIC CONTENTS AND ANTIOXIDANT ACTIVITIES OF *PTEROCARPUS SANTALINUS* LINN

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ABSTRACT: Plants and plant-based medicaments are the basis of many of the modern pharmaceuticals we use today for our various diseases. *Pterocarpus santalinus* Linn (Fabaceae) is a small to the medium-sized deciduous tree is widely distributed in the tropical regions of the world. The plant is mainly used to cure skin ailments, cough, hyper nervous activity and is also used as an anti-inflammatory, anti-cancer, and hepatoprotective agent. The present study aimed to determine qualitative and quantitative phytochemical and *in-vitro* antioxidant activities of the leaf of *P. santalinus*. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenol and flavonoids were determined by the well-known test protocol available in the literature. Quantitative analysis of phenolic and flavonoids was carried out by Folins Ciocalteau reagent and aluminum chloride methods, respectively. The *in-vitro* antioxidant activity of hydroalcoholic extract of the leaf was assessed against the DPPH assay method using standard protocols. Phytochemical analysis revealed the presence of flavonoids, diterpenes, phenol, protein, saponins, and tannins. The total phenolic and flavonoid content of *P. santalinus* leaves of the hydroalcoholic extract was 0.935 and 0.900mg/100mg respectively. The activities of hydroalcoholic leaves extract against DPPH assay method were concentration-dependent with IC_{50} values of ascorbic acid and extracted 14.23and 42.56µg/ml, respectively. These studies provided information for standardization and correct identification of this plant material. The diverse array of phytochemicals present in the plant thus suggests its therapeutic potential, which may be explored in drug manufacturing and traditional medicine.

INTRODUCTION: Plants as natural products are a valuable source of bioactive compounds and have been used for medicinal purposes worldwide. Recently, scientific attention to oriental medicine has increased in the context of discovering novel drugs for treating various diseases, including cancer and diabetes [1, 2]. The World Health Organization (WHO) endorses the evaluation of the potential benefits of plants as effective therapeutic agents, especially in areas where there is a lack of safe modern drugs [3]. One-third of total drugs (35%) in the USA and 80% of drugs used in fast-developing countries such as China and India are derivatives of phytoextracts [4, 5].

India has a rich heritage of medicinal plants of wide diversity, which are used by the local population and traditional healers to treat several diseases. Reactive oxygen species (ROS) such as superoxide anion, hydroxyl, hydrogen peroxide radicals, and peroxynitrite participate in inflammation in various tissues [6]. Excessively produced ROS can injure cellular biomolecules, such as nucleic acids, proteins, carbohydrates, and

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lipids, causing cellular and tissue damage, which in turn augments the state of inflammation [7, 8]. In addition to their role in acute inflammation, ROS may also contribute to several chronic cutaneous inflammatory diseases, such as psoriasis, atopic dermatitis, and contact dermatitis [9]. Therefore, compounds of natural origin that have scavenging activities toward these radicals may be expected to have therapeutic potentials for several inflammatory diseases. *P. santalinus* is a small to medium-sized deciduous tree that belongs to the family Fabaceae. It is widely distributed in tropical regions of the world. The plant has significant importance in traditional medicine for its ethnomedicinal value. The plant is mainly used to cure skin ailments, oral diseases, cough, pyrexia, diarrhoea, dysentery, hyper nervous activity and is also used as a potent anti-hemorrhagic, anti-inflammatory, anti-bacterial, anti-cancer, and hepatoprotective agent [10].

Posting *P. santalinus* from India to Europe originated in the 17th century, especially dyeing cloth [11]. Bioactive non-nutrient components of a plant determined in vegetable grains culmination are referred to as phytochemicals. Those Phytochemicals are crucial for the prevention of extreme issues [12]. Phytochemical investigations have revealed that *P. santalinus* contains triterpenes, flavones, coumarins, tannins, phenolic acids, polysterols, and essential oils. The active constituents of *P. santalinus* include alpha and beta santalol, Cedrol, pterocarpol, isopterocarpol, santalin A, B, pterocarpin, cryptomeridiol, and santalin [13]. The aim of this work was to determine the quality (types), quantity (amount) of bioactive compounds, and *in-vitro* antioxidant activity of the leaf of *P. santalinus*.

MATERIAL AND METHOD:

Plant Material: The leaves of *P. santalinus* were collected from Govt. Home Science College, Hoshangabad (Botany Department) in January 2021. Plant material (leaves) selected for the study were washed thoroughly under running tap water and then rinsed in distilled water; they were allowed to dry for some time at room temperature. Then the plant material was shade dried without contamination for about 3 to 4 weeks. Dried plant material was grinded using an electronic grinder. Powdered plant material was observed for their colour, odour, taste, and texture. Dried plant material was packed in air-tight container and stored for phytochemical and biological studies.

Chemical Reagents: All the chemicals used in this study were obtained from Hi-Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India), and SRL Pvt. Ltd. (Mumbai, India). All the chemicals and solvents used in this study were of analytical grade.

Defatting of Plant Material: 150 gram of dried powdered leaves of *P. santalinus* was shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place.

Extraction by Maceration Process: Defatted dried powdered has been extracted with hydroalcoholic solvent (70:30: Methanol: Water) using maceration process for 48 hrs. The extracts were evaporated above their boiling points and stored in an air-tight container free from any contamination until it was used. Finally, the percentage yields were calculated for the dried extracts.

Phytochemical Screening of the Extract: The extract of *P. santalinus* was subjected to qualitative analysis for the various phytoconstituents like alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, proteins, amino acids, and flavonoids [14, 15, 16].

Total Phenol Determination: The total phenolic content was determined using the method of Olufunmiso *et al.* [17]. A volume of 2ml of each extract or standard was mixed with 1 ml of Folin Ciocalteau reagent [18] (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate.

The mixture was allowed to stand for 15 min at room temperature. The colour developed was read at 765 nm using UV/visible spectrophotometer. The total phenolic content was calculated from the gallic acid's standard graph, and the results were expressed as gallic acid equivalent (mg/100mg).

Total Flavonoids Determination: The total flavonoid content was determined using the method of Olufunmiso *et al.* [17]. 1ml of 2% $AlCl_3$ solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer. The content of flavonoids was calculated using the standard graph of quercetin and the results were expressed as quercetin equivalent (mg/100mg).

DPPH Free Radical Scavenging Assay: DPPH scavenging activity was measured by modified method [19]. The spectrophotometer measured DPPH scavenging activity. Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. A decrease in the absorbance in the presence of sample extract at different concentrations (10-100 µg/ml) was noted after 15 min 1.5 ml of DPPH solution was taken, and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentrations were put in a series of volumetric flasks, and the final volume was adjusted to 3 ml with methanol.

Three test samples were taken, and each was processed similarly. Finally, the mean was taken. Absorbance at zero time was taken for each concentration. A final decrease in absorbance was noted of DPPH with the sample at different concentrations after 15 minutes at 517 nm. The percentage inhibition of free radical DPPH was calculated from the following equation:

% inhibition = [(absorbance of control - absorbance of sample) /absorbance of control] × 100%.

Though the activity is expressed as 50% inhibitory concentration (IC_{50}), IC_{50} was calculated based on the percentage of DPPH radicals scavenged. The lower the IC_{50} value, the higher the antioxidant activity.

RESULTS AND DISCUSSIONS: The percentage yields of petroleum ether and hydroalcoholic extract obtained from *P. santalinus* are depicted in **Table 1**. Preliminary phytochemical studies of the extract were done according to the published standard methods. These tests were broad in scope and used to determine the presence of flavonoids, diterpines, phenol, protein, saponins, and tannins. But alkaloids were absents in the extract **Table 2.** The content of total phenolic compounds (TPC) content was expressed as mg/100mg of the gallic acid equivalent of the dry extract sample using the equation obtained from the calibration curve: **y =** $0.014x + 0.004$, $R^2 = 0.999$, where X is the gallic acid equivalent (GAE), and Y is the absorbance. The content of total flavonoid compounds (TFC) content was expressed as mg/100mg of quercetin equivalent to a dry extract sample using the equation obtained from the calibration curve: $y = 0.021x + 0.008$, $R^2 = 0.999$, where X is the quercetin equivalent (QE), and Y is the absorbance. TPC of hydroalcoholic extract of *P. santalinus* showed the content values of 0.935and fallowed by TFC were 0.900 **Table 3**. Antioxidant activity of the samples was calculated through a DPPH assay. The % inhibition was calculated as an indication of antioxidant potency. The higher the % inhibition, the better the activity. Ascorbic acid was taken as standard, and the values were comparable with concentrations ranging from 10 µg/ml to 100µg/ml. A dose-dependent activity concerning concentration was observed in **Table 4** & **Fig. 1.**

TABLE 1: % YIELD OF LEAVES OF *P. SANTALINUS*

S. no.	Solvents	% Yield
1	Pet ether	0.260%
2.	Hydroalcoholic	10.97%

TABLE 2: PHYTOCHEMICAL SCREENING OF EXTRACT OF *P. SANTALINUS*

S. no.	Constituents	Hydroalcoholic extract
1.	Alkaloids: Hager's Test	-ve
2.	Glycosides: Legal's Test	-ve
3.	Flavonoids: Lead acetate Test: Alkaline test	+ve +ve
4.	Diterpenes: Copper acetate Test	+ve
5.	Phenol: Ferric Chloride Test	+ve
6.	Proteins: Xanthoproteic Test	+ve
7.	Carbohydrate: Fehling's Test	-ve
8.	Saponins: Froth Test	+ve
9.	Tannins: Gelatin test:	+ve

TABLE 3: TOTAL PHENOL AND TOTAL FLAVONOID CONTENT OF *P. SANTALINUS* EXTRACT

S. no.	Extract	Total Phenol (mg/100mg)	Total flavonoid (mg/100mg)
1.	Hydroalcoholic extract	0.935	0.900

TABLE 4: % INHIBITION OF ASCORBIC ACID AND HYDROALCOHOLIC EXTRACT USING DPPH METHOD

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Hydroalcoholic extract
1	10	41.93	10.94
2	20	56.45	31.38
3	40	61.29	56.93
4	60	72.58	77.37
5	80	75.8	83.21
6	100	80.64	85.4
	IC_{50}	14.23	42.56

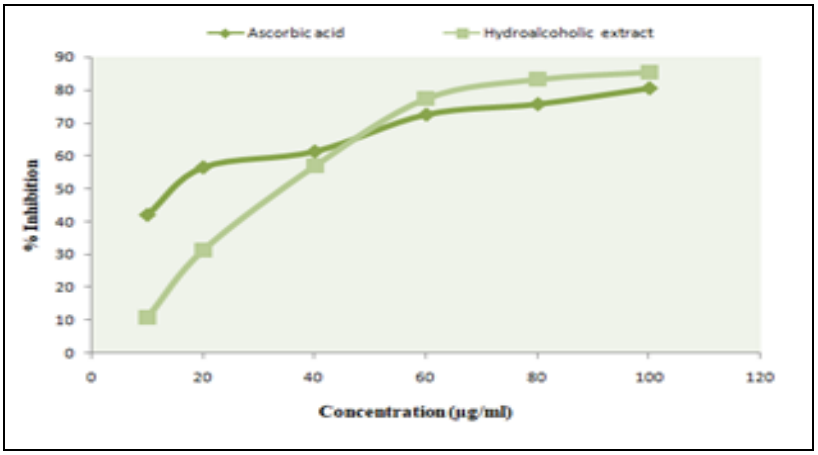

FIG. 1: % INHIBITION OF ASCORBIC ACID AND EXTRACT OF *P. SANTALINUS*

CONCLUSION: It can be concluded that from the present investigation, the phytochemical investigation gave valuable information about the different phytoconstituents present in the plant, which helps the future investigators concerning the selection of the particular extract for further investigation of isolating the active principle and also gave an idea about different phytochemicals have been found to possess a wide range of activities. The total phenolic and flavonoid content in hydroalcoholic leaf extract is further proved by *in-vitro* antioxidant studies. Potential antioxidant activity has good correlations with therapeutic use in treating cardiovascular disorders. Further research to isolate individual compounds and their in-vivo antioxidant activities with different mechanisms is needed.

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