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Analysis of Phytochemical Constituents and Evaluation of Invitro Anti-Inflammatory Activity of *Wrightia tinctoria* Leaf and Bark: A Traditional Medicinal Plant of India

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Abstract

In this present study, the phytochemical constituents and anti-inflammatory activity of *Wrightia tinctoria* leaf and bark samples are estimated. The solvents such as acetone, aqueous, ethanol, and methanol are added with leaf and bark extracts of *W. Tinctoria* plant and evaluated. The preliminary screening test is executed for the presence of secondary metabolites and was reported in order to understand the phytochemicals of leaf and bark. Both qualitative and quantitative analysis of alkaloids, coumarins, flavonoids, phenols, proteins, saponins, steroids, tannins, terpenoids, and quinones were done. Each extracted solvents are found to be recorded with alkaloid content. Aqueous and methanol extracts of *W. tinctoria* leaf and bark have been tested for their ability to replicate anti-inflammatory action. The medicinal plant *W. tinctoria* leaf and bark extract with aqueous solvent gave excellent anti-inflammatory properties when compared with methanolic solvents of bark and leaf at 500 µg/ml concentration respectively. Obviously, *W. tinctoria* aqueous leaf extract showed extraordinary anti-inflammatory activities. Hence the medicinal plant *W. tinctoria* is more suitable as a drug for biomedical properties in the human era.

***Key words*:** *Wrightiat inctoria,* Leaf, Bark, Solvents, Phytochemicals, Anti-inflammatory

Pathogenicity of microbes and additional infectious diseases include restrictions through the use of commercially existing antimicrobial drugs meant for the last several years. Enormous usage of drugs has built-up multiple drug resistance (MDR) in numerous pathogens of bacteria. As a alter for this at the moment, natural plants have been the foundation of lots of conventional drug systems all through the world and sustained to provide humankind by novel remedies. A massive range of therapeutic plants, their purified bioactive, and their products as of the therapeutic plants are provided with infinite impunities intended for novel drug development because of the incomparable ease of access of diverse chemical compounds. They are habitual of inexpensive having less adverse effects and improved efficiency in MDR outbreaks. India is solitary of the nation that comprehensively makes use of herbal drugs to meet the requirements of healthcare and it is used to change the commercial antibiotics. Phytodrugs reveal their remedial abilities via an assortment of bioactive components such as alkaloids, saponins, carbohydrates, glycosides, flavonoids, terpenoids, gums, steroids, phenolic compounds, volatile oils, etc. derived from abundant species of therapeutic plants. Diverse parts of these therapeutic plants are conventionally used as an ayurvedic drugs in diverse regions [1].

Traditional herbal and medicinal plant products have been used by humans since the beginning of the human race. The green land of India has a prosperous flora of diverse plants and it is used in habitual therapeutics [2]. *W. tinctoria* is a widely utilized traditional medicinal plant in India. Genus of *Wrightia* is named by a Scottish physician also botanist William Wright. The foliage of this tree gives a blue dye known as pala indigo. It is known by common name as “indrajav” [3-5]. The family Apocynaceae is distributed in Central India, Burma, and Timor [6]. *W tinctoria* is a small deciduous tree widely found throughout various parts of India. The tree is used for enormous medicinal purposes. The leaves are used in Ayurvedic medicines for treating toothache and hypertension; the bark and seeds are used to treat various indigestion and skin problems. The leaves are especially useful in treating skin diseases. Also, this plant is very useful as stomachic, in the treatment of abdominal pain, skin diseases, anti-diarrhoeal and anti-

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haemorrhagic. For the treatment of psoriasis, they are used widely in Siddha medicine. From the leaves of *W. tinctoria*, the major constituents such as indirubin, indigotin, isatin, tryptanthrin, rutin and anthranilate are isolated. They also contain lupeol, β-amyrin, ursolic acid, and β-sitosterol. The leaves are found to be acrid whereas the bark and seed are found to be bitter. The leaves have thermogenic and hypotensive properties. The bark and seeds have carminative, thermogenic, depurative, anthelmintic, and aphrodisiac properties.

There are various techniques that involve different cost and level of complexity in the extraction of plant material and the choice of the better solvent in extraction of those plant materials is one of the important steps. The factors such as safety, solubility, grade, ease of working, and purity should be considered while selecting the solvent for extracting the plant materials. The phytochemistry or plant chemistry is one of the recent subjects developed as a distinct discipline, which lies in between organic chemistry and plant biochemistry. The role of secondary metabolites is previously underestimated since they do not get much involved into the plant's primary metabolism. The varied roles of these groups of compounds are realized in recent years. The chemical profile of the plants can be analyzed using certain qualitative chemical tests. This paper is highlighted to record the pharmacological constituents of the traditional medicinal plant *W. tinctoria*. Here, *W. tinctoria* leaf and bark extracts were prepared using several solvents, succeeded by identifying the chemical constituents, and determining the anti-inflammatory activity with the aim of finding its suitability for utilization in biomedical fields [7].

MATERIALS AND METHODS

Collection of plant

Fresh leaves of *W. tinctoria* along with bark were collected during the right season from the agricultural land near Chittode in Thanjavur, Tamilnadu, India. The taxonomic identity of the plant was confirmed as *W. tinctoria* R. Br. belonging to the family Apocynaceae under the Botanical Survey of India, Tamil Nadu.

Preparation of leaf powder

The collected fresh leaves of *W. tinctoria* were washed well using running water and also by double distilled water. This helped to remove all impurities present in the collected leaves. They were then dried under shade for a period of 20 days to remove the excess moisture present in them and this process helps to avoid destruction of active compounds. After drying, they were ground entirely into fine powder and stored in airtight containers for further study.

Sample preparation

10gm of the dried leaf and bark powder of *W. tinctoria* were taken separately in labelled airtight bottles and 50 ml of each solvent such as acetone, aqueous, ethanol, and methanol were individually added. The solvents help to penetrate the tissues of the leaves and bark to dissolve its active principles.

Qualitative phytochemical analysis

The investigation of the drugs comprises the study of phytochemicals acquired from the plants. Qualitative phytochemical study assists to establish the profile and chemical composition of the extracts [8]. It was done to assess the qualitative chemical composition of crude extracts using commonly employed, precipitation and colorations reactions to identify the major natural chemical groups such as alkaloids, coumarins, flavonoids, phenols, protein, quinine, saponins, steroids, tannins, and terpenoids. General reactions in this analysis revealed the presence or absence of these compounds in the plant extracts. The extracts were tested for the existence of several phytochemicals as per the methods given by Harborne.

Test for alkaloids

Mayer's test: $Hgcl_2$ (1.36 gm), KI (5 gm) were disbanded in 60 ml and 10 ml of refined water respectively. The above-mentioned solvents were assorted were watery to l00 ml using refined water. Scant droplets of reagent were supplemented to one ml of peel extract and it creates the colorless precipitate indicating the existence of alkaloids [7].

Test for coumarins

0.5 g of the moistened extracts was taken in a test tube. The mouth of the tube was covered with filter paper treated with 1N NaOH solution. In boiling water the test tube was placed for few minutes afterwards the filter paper was isolated and examined under the UV light for the presence of coumarins which is indicated by yellow fluorescence [9].

Test for flavonoids

To 1ml of the leaf extract, a few drops of dilute sodium hydroxide was added. An intense yellow colour appeared in the plant crude extract, which became colourless on the addition of a few drops of dilute acid which indicates the presence of flavonoids [10].

Test for phenols

In the $Fecl_3$ test, 1ml extract was supplemented with refined water 2 ml, afterward add few drops of 10% aqueous $Fecl_3$ solution. Blue or green precipitate is confirmed as the subsistence of phenols [7].

Test for proteins

Biuret test: Two drops of 3% copper sulphate and few drops of 10% sodium hydroxide were added to 2ml of leave extract. The presence of proteins was indicated by the formation of a violet or red colour [11].

Test for Quinones

2 ml of conc. H_2SO_4 was added to 1 ml of leave extract. The presence of quinone was indicated by the formation of a red colour [12].

Test for saponins

2 ml distilled water was added to 2 ml leave extract and shaken for 15 minutes lengthwise in a graduated cylinder. Saponin was detected by the formation of a 1cm layer of foam [13].

Test for steroids

Liebermann-burchard reaction: In chloroform a few crystals were dissolved and a few drops of conc. H_2SO_4 were added to it and it was followed by the addition of 2-3 drops of acetic anhydride solution. Finally, the solution changes into green from violet colour.

Test for tannins

5 ml of the *W. tinctoria* extract was placed in a test tube and then 2 ml of 5% of $FeCl_3$ solution was added. The presence of tannins is indicated by blue or black precipitate.

Test for terpenoids

Salkowski test

5 ml of various solvent extract was mixed in 2 ml of chloroform followed by the careful addition of 3 ml concentrated (H_2SO_4). A positive result for the presence of terpenoids is indicated by the formation of a layer of the reddish brown colour at the boundary.

Quantitative phytochemical analysis

Estimation of alkaloids

Alkaloid determination by using Harborne method [14].1g of the *W. tinctoria* leaf and bark was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and it's covered and allowed to stand for 4 hrs. Extract was filtered and then it was allowed to stand on a water bath upto one quarter of the original volume arrives. Concentrated NH_4OH was added by drop wise to the extract until the precipitation was completed. The entire solution was allowed to settle and the precipitate was collected and washed with dilute NH_4OH and then it was filtered. The residue obtained is alkaloids which was dried and weighed.

$$\text{Gram in \% of alkaloid} = \frac{\text{Weight of alkaloid}}{\text{Weight of sample used}} \times \frac{100}{1}$$

Estimation of coumarins

Leaf and bark powder (2.5 g) was added to a beaker containing 25 ml of acetone, aqueous, ethanol, methanol and placed in a water bath shaker, adjusted at 37°C for 24 hours. The extracts were filtered using Whatmann No.1 filter paper and the resulted solutions were concentrated under reduced pressure and weighed. Coumarins stored in amber tightly-closed containers are apparently labelled and kept in the refrigerator for further phytochemical analysis.

$$\text{Gram in \% of coumarins} = \frac{\text{Weight of coumarins}}{\text{Weight of sample used}} \times \frac{100}{1}$$

Estimation of flavonoids

2g of *W. tinctoria* sample leaf and bark was repeatedly extracted with 100 ml of 80% acetone, aqueous, ethanol, and methanol at room temperature. The mixture was filtered through a Whatmann No.1 filter paper into a pre-weighed 250 ml beaker. The filtrate was transferred into a water bath and allowed to evaporate to dryness and weighed [13].

$$\text{Gram in \% of flavonoids} = \frac{\text{Weight of coumarins}}{\text{Weight of sample used}} \times \frac{100}{1}$$

Estimation of Phenols

The fat-free samples were boiled with 50 ml of ether for the extraction of the phenolic compound for 15 min. 5 ml of the respective extract was pipetted out into a 50 ml flask, then 10 ml of distilled water was added. 2 ml of NH_4OH solution and 5 ml of concentrated amyl alcohol were also added. The plant leaf and bark samples were made up to mark and left to react for 30 min for colour development. The absorbance was read at 505 nm [14].

$$\text{Phenol (mg/100g)} = \frac{\text{Abs. of sample}}{\text{Abs. of std}} \times \frac{\text{Conc. of std}}{\text{Wt. of sample}} \times \text{dilution factor} \times \frac{100}{1}$$

Estimation of protein

By using Bradford's method, the total protein content was determined. 100 µl of the respective samples extract added 3 ml of Bradford's reagent and incubated in dark for 5 minutes. The absorbance was measured at 595nm. Bovine serum albumin dilutions (0.1mg/ml 0.5mg/ml) are used as standard solutions [15].

$$\text{Gram in \% of protein} = \frac{\text{Weight of protein}}{\text{Weight of sample used}} \times \frac{100}{1}$$

Estimation of quinone

A test portion of the powder containing 100 mg of Quinone sulfate was dissolved in 100 ml of 0.1 N HCl. A dilution of 5 ml in 100 ml of 0.1 N HCl was carried out after homogenization. Using spectrophotometer, the absorbance was read at 348 nm. The reference product was treated in the same way and under the same conditions [16].

$$\text{Gram in \% of quinone} = \frac{\text{Weight of quinone}}{\text{Weight of sample used}} \times \frac{100}{1}$$

Estimation of saponins

The *W. tinctoria* samples leaf and bark individually were ground. 20g of each plant sample respective were dispersed in 200 ml of 20% ethanol. At about 55°C with continuous stirring the suspension was heated in a water bath for 4 hours. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. At about 90°C, over water bath the combined extracts were reduced to 40 ml. The concentrated samples were transferred into a 250 ml separator funnel and 20 ml of diethyl ether was added and shaken vigorously. The ether layer was discarded also the aqueous layer was recovered. The purification process was repeated. 60 ml of n-Butanol was added. Using 10 ml of 5% aqueous sodium chloride the combined n-Butanol extracts solution was washed twice. The remaining solution obtained was heated using water bath. After evaporation, the respective samples were dried in the oven to a constant weight. The percentage of saponins content was calculated [17].

$$\text{Gram in \% of saponins} = \frac{\text{Weight of saponins}}{\text{Weight of sample used}} \times \frac{100}{1}$$

Estimation of total steroids

1gm extract was macerated with 20 ml of ethanol and filtered. 2 ml of chromogen solution was added and the solution was left to stand for 30 min. The absorbance was read at 550nm [14].

$$\text{Gram in \% of steroids} = \frac{\text{Weight of steroids}}{\text{Weight of sample used}} \times \frac{100}{1}$$

Estimation of tannins

500 mg of the *W. tinctoria* was weighed into a 50 ml plastic bottle. 50ml of solvent was added and shaken for 1 hrs in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then into a test tube 5 ml of the filtrate was pipette out and then it was mixed with 2 ml of 0.1M $FeCl_3$ in 0.1 N HCL and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 mm. The assessment of the tannin content was done in triplicate. The tannin content was expressed in terms of mg of tannic acid equivalents/grams of the dried sample [14].

$$\text{Tannin (mg/100g)} = \frac{\text{Abs. of sample}}{\text{Abs. of std}} \times \frac{\text{Conc. of std}}{\text{Wt. of sample}} \times \text{dilution factor} \times \frac{100}{1}$$

Estimation of terpenoids

Dried *W. tinctoria* leaf and bark extract of 100mg was taken and soaked in 9 ml of methanol and aqueous for 24 hours. After filtration using a separating funnel, the extracted solution was treated with 10mL of petroleum ether. The plant ether

extract was separated in pre-weighed glass vials and waited for its complete drying (wf) was evaporated and the yield (%) of total terpenoids contents was measured by the following formula (wi-wf / wi × 100) [14].

In vitro anti-inflammatory activity

Inhibition of albumin denaturation

The reaction mixture consists of test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount of HCl. The extracted sample were incubated at ambient temperature for 20 min later it was heated to 51°C for 20 min. After cooling the samples, turbidity was measured spectrophotometrically at 660 nm [18]. The experiment was performed in triplicate. Percent% inhibition of denaturation of protein was evaluated as follows:

$$\text{Percent\% inhibition} = [\{\text{Abs control} - \text{Abs sample}\}/\text{Abs control}] \times 100$$

Anti-Inflammatory compounds

W. tinctoria medicinal plants is reviewed for their phytochemical compositions and pharmacological properties, this plant species were studied for its phytochemistry. When compounds isolated from *W. tinctoria* medicinal plants it were further reviewed, for various anti-inflammatory activities in invitro cellular models. In the anti-inflammatory compounds majority were terpenes and terpenoids, followed by flavonoids, coumarins, alkaloids, glycosides, sterols, lignans, and carboxylic acids. The rest of the compounds were phenolics, aldehydes, tannins, pyrans, phenylpropanoids, and fatty acid esters [19].

Statistical analysis

Experiments were carried out in triplicate and the results are expressed as mean values with standard deviation.

RESULTS AND DISCUSSION

Estimation of qualitative phytochemical constituents of *W. tinctoria*of leaf extraction with different solvents like acetone, aqueous, ethanol, and methanol were performed. The maximum phytochemicals such as alkaloids, coumarins, flavonoids, Phenols, steroids, tannin and terpenoids were estimated in leaf of *W. tinctoria* with all solvents respectively and absents of qualitative phytochemical constituents such as protein and saponins in acetone extract, quinines in aqueous extract, quinones and saponins in ethanolic extract and protein from methanol extract were analysed some phyto compounds profuse from *W. tinctoria* leaf extracts respectively. The percentage of extract after soxhlation was found to be 1.33% for *W. tinctoria* respectively. The phytochemical screening and analysis of medicinally important species showed that leaf and barks were rich in steroids, terpenoids, fatty acids, tannins, saponins, coumarins, and emodins. Flavonoids, phenols, and alkaloids were also present [20]. The results of the present study have showed that the leaf and bark of *W. tinctoria* could be explored in the management of pain, inflammation fever and its effect may be due in part to the phytoconstituents, especially the flavonoid content [21-22] (Table 1).

Analysis of qualitative phytochemical constituents of *W. tinctoria* of bark extraction with different solvents like acetone, aqueous, ethanol, and methanol were performed. The maximum phytochemicals such as alkaloids, coumarins, flavonoids, Phenols, protein, steroids, tannin, and terpenoids were estimated in the bark of *W. tinctoria* with aqueous solvents and alkaloids, Phenols, Quinones, Saponins, steroids, tannin, and terpenoids in methanolic extract respectively. Minimum qualitative phytochemical constituents such as alkaloids, coumarins, protein, and quinones are presented in the acetone extract, and alkaloids, protein, saponins, steroids and tannin are presented in ethanolic extract of *W. tinctoria* bark extracts (Table 1).

Table 1 Qualitative analysis of phytochemical compounds from *Wrightia tinctoria*

Phytochemical compounds	Leaf extract				Bark extract			
	Acetone	Aqueous	Ethanol	Methanol	Acetone	Aqueous	Ethanol	Methanol
Alkaloids	+	+	+	+	+	+	+	+
Coumarins	+	+	+	+	+	+	-	-
Flavonoids	+	+	+	+	-	+	-	-
Phenols	+	+	+	+	-	+	-	+
Protein	-	+	+	-	+	+	+	-
Quinones	+	-	-	+	+	-	-	+
Saponins	-	+	-	+	-	-	+	+
Steroids	+	+	+	+	-	+	+	+
Tannin	+	+	+	+	-	+	+	+
Terpenoids	+	+	+	+	-	+	-	+

(+) Present, (-) absent

Differential analytical determination of *W. tinctoria* leaf phytochemicals at maximum level of ethanolic and aqueous extract was (15.3±0.69 and15.2±4.04 mg/g) protein were founds to be recorded respectively whereas the maximum level and minimum level of quinones (15.8±3.05 and 7.02±0.36 mg/g) in methanolic and acetone extract can be recognized in the quantity level of compounds respectively (Table 2). This results in line with [23]. The phytochemical evaluation carried for therapeutic plants revealed that alkaloids, saponins, terpenoids, phenols, and anthraquinones were commonly found in all the extracts. The crude methanolic extract of *Pseudocaryopteris foetida* leaves showed strong positive results for the presence of tannins, phenolics, flavonoids and terpenoids. While, the fractions including n-hexane fraction, ethyl-acetate fraction, and methanol showed strong positive results only for phenolic and flavonoids. The quantitative analysis of *P. foetida* leaves methanol extract was rich in phenols (163.5 ± 1.2 mg/g), flavonoids (37.54 ± 0.7 mg/g), and tannin (17.5 ± 1.7 mg/g) as explored in [24]. In the present study phytochemical constituents that report biological active nature to the plant were analysed and results confirmed maximum phytochemicals such as alkaloids, coumarins, flavonoids, Phenols, steroids, tannin and terpenoids were estimated in leaf of *W. tinctoria* with all the solvents.

Table 2 Quantitative analysis of phytochemical compounds from *Wrightia tinctoria* leaf extract

Phytochemical compounds	Quantity (mg/g)			
	Acetone	Aqueous	Ethanol	Methanol
Alkaloids	12.8±0.03	12.5±0.05	12.4±0.05	11.4±0.03
Coumarins	11.0±0.05	10.0±0.06	10.7±0.05	10.9±0.04
Flavonoids	10.8±0.06	11.7±0.07	9.70±0.04	11.0±0.02
Phenols	13.0±0.03	11.3±0.10	10.6±0.01	11.5±0.37
Protein	-	15.2±4.04	15.3±0.69	-
Quinones	7.02±0.36	-	-	15.8±3.05
Saponins	-	11.2±1.00	-	8.00±0.65
Steroids	12.9±0.03	12.4±0.01	10.7±0.03	11.4±0.00
Tannin	12.4±0.13	10.6±0.16	12.5±0.25	12.2±3.56
Terpenoids	10.6±0.03	9.78±0.05	10.5±0.25	10.4±0.09

Standard deviation ± error

Differential analytical determination of *W. tinctoria* leaf phytochemicals at maximum level of aqueous extract was (15.2±4.04mg/g) protein founds to be recorded respectively whereas in minimum level of quinones (7.02±0.36 mg/g) in acetone extract can be recognized in the quantity level of compounds respectively (Table 2). As per the quantitative phytochemicals of bark extract of *W. tinctoria* with aqueous extract was protein (14.0±0.63mg/g) and minimum phytochemical of the quantity of quinones (6.20±0.20mg/g) in acetone extract are revealed with respective plant (Table 3). The methanol and aqueous extracts of *W. tinctoria* were studied for *in vitro* anti-inflammatory activity by HRBC membrane stabilization method. Phytochemical investigation reveals that methanol extracts contains carbohydrates, steroids, alkaloids, terpenoids, flavonoids, tannins, polyphenols while aqueous extract contains carbohydrates, alkaloids, flavonoids, tannins, polyphenols. *T. terrestris* dichloromethane, methanol, and 70% aqueous methanol extracts were examined for *in vivo* anti-inflammatory activities against carrageenan and formaldehyde-induced paw edema in rats and mice, respectively. Methanol extract outlined significant inhibition of carrageenan- (69.3%, $p < 0.001$) and formaldehyde- (71.3%, $p < 0.001$) induced paw swelling at 200 mg/kg b.w., after 3 and 24 h, separate when compared to control [25-26].

Table 3 Quantitative analysis of phytochemical compounds from *Wrightia tinctoria* bark extract

Phytochemical compounds	Quantity (mg/g)			
	Acetone	Aqueous	Ethanol	Methanol
Alkaloids	12.7±0.49	10.8±0.06	10.6±0.05	10.7±0.06
Coumarins	11.0±0.02	10.8±0.02	-	-
Flavonoids	-	11.9±0.08	-	-
Phenols	-	10.4±0.01	-	10.8±0.04
Protein	11.8±0.01	14.0±0.63	12.0±0.13	-
Quinones	6.20±0.20	-	-	11.0±0.00
Saponins	-	-	10.4±0.42	10.9±0.78
Steroids	-	10.0±0.03	10.7±0.05	10.5±0.03
Tannin	-	9.06±0.00	9.00 ±0.00	9.20±0.00
Terpenoids	-	13.9±0.03	-	9.73±0.02

Standard deviation ± error

Table 4 Anti-inflammatory activity of *Wrightia tinctoria* with different concentration

Plant extract different concentration (µg/ml)	(%) percentage of inhibition			
	Leaf		Bark	
	Aqueous	Methanol	Aqueous	Methanol
100	18.7±0.82	14.5±1.26	14.1±0.26	15.0±6.35
200	19.5±2.84	15.1±1.20	16.9±2.59	15.8±0.48
300	21.1±2.15	16.7±3.66	17.0±5.09	16.4±5.27
400	23.4±5.59	18.1±0.87	18.0±0.81	18.6±2.82
500	23.9±4.48	19.9±7.00	19.5±7.50	18.9±0.52

Standard deviation ± error

In the present study, it was found that the inhibition rate of aqueous extract, Methanol extract and gradually increased with the increased in the concentration. It was found that 500 µg/ml of *Wrightia tinctoria* leaf aqueous extract showed the highest anti-inflammatory activity. Analysis of anti-inflammatory activities of *Wrightia tinctoria* leaf with aqueous extract of different concentration of 100, 200, 300, 400 and 500 µg/mL was 18.7±0.82, 19.5±2.84, 21.1±2.15, 23.4±5.59 and 23.9±4.48% of inhibition exhibited respectively, The methanolic extract of 14.5±1.26, 15.1±1.20, 16.7±3.66, 18.1±0.87 and 19.9±7.00mg/g. *Wrightia tinctoria* bark with aqueous extract 14.1±0.26, 16.9±2.59, 17.0±5.09, 18.0±0.81 and 19.5±7.50 mg/g, the methanolic extract of 15.0±6.35, 15.8±0.48, 16.4±5.27, 18.6±2.82 and 18.9±0.52 % of inhibition exhibited respectively (Table 4). The Concentration range of MKI (Mudakkaruththaan ilakam) at 100, 200, 300, 400 and 500 µg /ml produces significant inhibition of protein denaturation in concentration dependent manner. The inhibitory effect of different concentrations of MKI on protein denaturation as showed. Maximum percentage of inhibition is about 41.7±7.414% and it was observed at 500 µg/ml when compared to diclofenac sodium, a standard anti-inflammatory agent with the maximum inhibition 93.76±4.782 at the concentration of 100µg/ml. The result obtained from the study indicates that the

test drug MKI was effective inhibiting heat induced albumin denaturation [7]. The current study also provides strong evidence for the use of the leaf *Wrightia tinctoria* it was found that 500 µg/ml of leaf aqueous extract showed the highest anti-inflammatory activity. In leaf extract the anti-inflammatory activity of *Wrightia tinctoria* at 500µg/ml showed 23.9±4.48 in aqueous and 19.9±7.00 in methanolic extract. Also, in bark extract the anti-inflammatory activity of *Wrightia tinctoria* at 500 µg/ml showed 19.5±7.50in aqueous and 18.9±0.52 in methanolic extract respectively.

CONCLUSION

The present study revealed that *Wrightia tinctoria* part of leaf and bark is an important medicinal plant with diverse phytochemical constituents. The plant showed the presence of numerous chemical constituents like alkaloids, coumarins, flavonoids, phenols, protein, quinine, saponins, steroids, tannins, and terpenoids which are responsible for the various pharmacological and medicinal property like Anti-inflammatory activity. However, evaluation needs to be carried out on *Wrightia tinctoria* that *in vitro* production of secondary metabolites of these phytochemicals can be formulated for drug development in future and regulate the system for the forthcoming generation.

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