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ESTIMATION OF PHYTOCHEMICALS AND QUANTITATIVE STUDY PHENOLIC AND FLAVONOIDS CONTENT IN LEAVES EXTRACT OF *WRIGHTIA TINCTORIA*

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ABSTRACT

Considering the vast reach of traditional medicine and a growing interest in self-produced medicines, the use of therapeutic plants is growing globally. One such plant *Wrightia tinctoria* R.Br. (Family: Apocynaceae), sometimes known as "Indrajau," is found all over the world and is particularly common in India. Traditionally it is used for many purposes. This study focuses on analysing it phytochemicals qualitatively & quantitatively. The leaves of plant were gathered & subjected to extraction by hydroalcoholic solvent. Qualitative & quantitative tests were then performed according to standard protocol. Results revealed that plant contain flavonoids, phenol, proteins, carbohydrates, saponins. The Rf value for quercetin standard was found to be 0.52. The bands obtained for long, short & normal light are 5,2 & 1 respectively. From the Rf value obtained from the extract it can be interpreted that extract contain range of flavonoid compounds along with quercetin. Total phenol & flavonoid content in *Wrightia tinctoria* extract was found to be 0.241 mg/100mg & 0.775 mg/100mg respectively. Thus, obtained results point towards fact that *Wrightia tinctoria* contain ample types & amounts of phytochemicals.

Keywords: Medicinal plants, Phytochemicals, Herbal medicines, *Wrightia tinctoria*, Phenol, flavonoids, TLC.

INTRODUCTION

The presence of phytochemicals in medicinal plants makes them effective for both treating and curing human ailments. The best source of valuable molecules for developing new medications for numerous major ailments like cancer, tumours, AIDS, and other human disorders is medicinal plants. It is well known that medicinal plants produce specific chemicals that prevent bacterial or fungal growth. Due to the build-up of bioactive phytochemicals in plant tissue, which are classified as primary and secondary metabolites, plants offer therapeutic effects [1,2].

In contrast to macronutrients and micronutrients, phytochemicals are biologically active, naturally occurring chemical compounds that are present in plants. They provide health benefits for people that

cannot be linked to these nutrients. In order to give defense against environmental variables and pathogenic agents in plants, these particular chemicals are produced by primary or rather secondary metabolism of living organisms. Secondary metabolites' biological effects are not just limited to the defense mechanisms of plants; they have also been employed to treat a number of human ailments. Organic substances found in plants called secondary metabolites have defined physiological effects on humans. A comprehensive range of therapeutic activity, including antibacterial, antiviral, immunomodulatory, anti-inflammatory, and most extensive anticancer activity, is known to be present in plant active bio constituents. Tanning agents, alkaloids, sugars, terpenoids, steroids, and flavonoids are some of these bioactive compounds. These can be made from fruits, seeds, barks, leaves, flowers, roots, or blossoms [3,4].

The biological effects of phenolics, which are strong antioxidants and free radical scavengers, have been the subject of several investigations. Because of their ability to function as reducing agents, hydrogen donors, and singlet oxygen quenchers due to their redox characteristics, phenolics have significant antioxidant activity. Therefore, there is growing interest in phenolic compounds obtained from plants and their functions in nutrition. Additionally, phenolic chemicals are well known for stabilising lipids against peroxidation and blocking a variety of oxidising enzymes [5,6].

Wrightia tinctoria R.Br. (Family: Apocynaceae), sometimes known as "Indrajau," is found all over the world and is particularly common in India. In traditional medicine, this plant's bark is used as a galactagogue to heal wounds, skin conditions, and gastrointestinal problems. To treat herpes and the mumps, the leaves are used as a poultice. They are occasionally also consumed to treat toothaches. This plant's seeds are also employed as an aphrodisiac. According to studies, W. tinctoria pod oil emulsion is used to cure psoriasis. Additionally, the bark of W. tinctoria has demonstrated the previously described anti-nociceptive, immunomodulatory, and wound healing properties [7-9]. Based on above findings this work focuses on estimating bioactive constituents of *W. tinctoria.*

MATERIAL AND METHODS

Collection of plant material

Leaves of *Wrightia tinctoria* was collected from ruler area of Bhopal (M.P), India in the months of February, 2023.

Extraction by maceration process

Following procedure was adopted for the preparation of extract from the shade dried and powdered herbs. Dried leaves of *Wrightia tinctoria* were extracted with hydroalcoholic solvent (Ethanol: water: 70:30v/v). The extract was evaporated above their boiling points. Finally, the percentage yields were calculated of the dried extract.

Phytochemical tests

The screening for phytochemical test were performed as per the standard methods.

Thin layer chromatography

Thin Layer Chromatography is a technique used to isolate non-volatile mixtures. The experiment is conducted on a sheet of aluminium foil, plastic, or glass which is coated with a thin layer of adsorbent material. The material usually used is aluminium oxide, cellulose, or silica gel. The Rf value was then calculated which will further help in qualitative & quantitative estimation of phytochemical [10].

Total phenol content estimation

The total phenol content of the extract was determined by the modified folin-ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10- 50µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer [11].

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method (Gaur Mishra *et al.*, 2017). 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids.1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm [12].

RESULTS & DISCUSSION

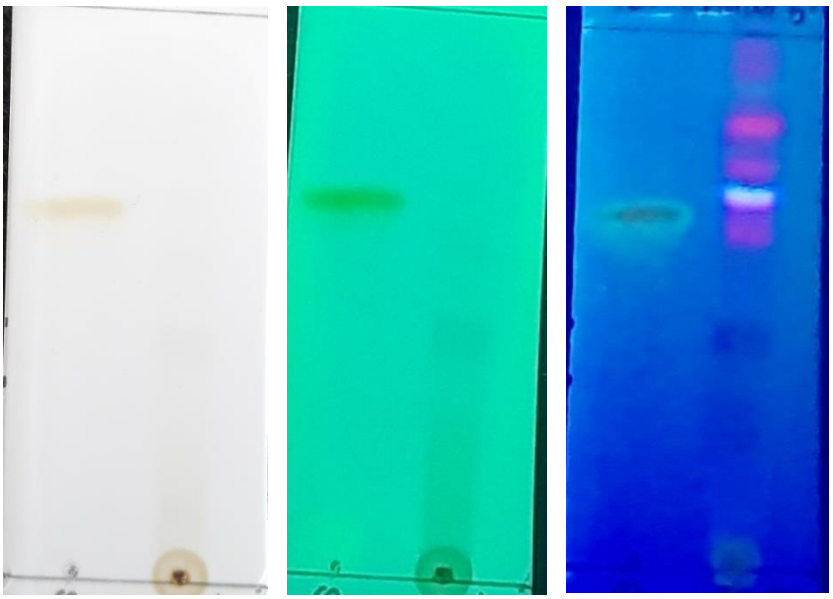
The results of phytochemical screening confirmed the presence of flavonoids, phenol, proteins, carbohydrates, saponins. Further Thin layer chromatography was also performed specifically for flavonoids. The Rf value for quercetin standard was found to be 0.52. The bands obtained for long, short & normal light are 5,2 & 1 respectively. From the Rf value obtained from the extract it can be interpreted that extract contain range of flavonoid compounds along with quercetin. Total phenol & flavonoid content in *Wrightia tinctoria* extract was found to be 0.241 mg/100mg & 0.775 mg/100mg respectively.

Table 1: Result of phytochemical screening of *Wrightia tinctoria* extract

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids**	
	Wagner's test	-ve
	Hager's test	-ve
2.	**Glycosides**	
	Legal's test	-ve
3.	**Flavonoids**	
	Lead acetate	+ve
	Alkaline test	+ve
4.	**Phenol**	
	Ferric chloride test	+ve
5.	**Proteins**	
	Xanthoproteic test	+ve
6.	**Carbohydrates**	
	Fehling's test	+ve
7.	**Saponins**	
	Foam test	+ve
8.	**Diterpenes**	
	Copper acetate test	-ve
9.	**Tannins**	
	Gelatin Test	-ve

Table 2: TLC of *Wrightia tinctoria* extract

	***Wrightia tinctoria* extract**	
S. No.	**Mobile phase** Toluene: Ethyl acetate: Formic acid (5:4:1)	***Rf* value**
1.	**(Quercetin)**	
	Dis. travel by mobile phase= 5.5cm	
	No. of spot at long UV= 1	Long- 0.52
	No. of spot at short UV = 1	Short- 0.52
	No. of spot at normal light= 1	Normal- 0.52
2.	**(Extract)**	
	Dis. travel by mobile phase= 5.5cm	
	No. of spot at long UV = 5	Long- 0.44, 0.52, 0.58, 0.78,0.82
	No. of spot at short UV = 2	Short- 0.24, 0.46
	No. of spot at normal light= 1	Normal- 0.56
	Spot Sequence	
	Quercetin	1st
	Extract	2nd



Normal Light **Short UV** **Long UV**

Table 3: Total bioactive constituents' content of *Wrightia tinctoria*

S. No.	Extract	Total phenol (mg/100mg)	Total flavonoid (mg/100mg)
1.	Hydroalcoholic extract	0.241	0.775

CONCLUSION

The current study found that *Wrightia tinctoria*, a plant with significant medicinal value, had good yields in a variety of TPC and TFC assays. According to early phytochemical analysis of *Wrightia tinctoria* leaf hydroalcoholic extracts, the presence of flavonoids, phenol, proteins, carbohydrates, and saponins could be the cause of these plant therapeutic efficacy. In order to investigate hidden aspects of *Wrightia tinctoria* and their practical clinical application, which can be used for the welfare of mankind port in carrying out this study at the laboratory, more analysis needs to be done.

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