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Analysis of phytochemical constituents and antibacterial activity of *Wrightia tinctoria*: traditional medicinal plant of India for application on wound dressing materials

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Wrightia tinctoria, an important traditional medicinal plant is exploited for treating several diseases. The study intends to reveal the presence of phytochemicals and test the antibacterial activity of *W. tinctoria* leaf extracts on nonwoven fabrics to find its suitability for wound dressings. The methodology includes identification and collection of *W. tinctoria* leaves, preparation of leaf powder, determination of physicochemical analysis, extraction using different solvents, preliminary phytochemical screening, quantitative estimation of phytoconstituents, yield of the extracts and determination of antibacterial activity on plant extract treated fabrics. Results revealed the presence of more active metabolites in the ethanol plant extract of *W. tinctoria* leaves which may be the reason for the promising antibacterial potential against the bacterial strains. As a promising ethnomedicinal plant, *W. tinctoria* may serve as a major source of useful drugs finding its suitability for developing wound dressings.

Keywords: Antibacterial activity, Phytochemical screening, Soxhlet extraction, Wound dressings, *Wrightia tinctoria*

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Herbal medicines are used not only for the treatment but also for the prevention of diseases and have become popular over the past few years. Unlike the western medicines which have more side effects, they are found to be effective and safe and so people are turning to herbal medicines. Plants are known for the traditional use of medicine having more therapeutic importance. They are considered as one of the best sources for drug preparation[1]. The improved progress of civilization and also the development in human knowledge makes use of the plants for medicine. Various chemical constituents are isolated from the plants by the scientists to perform biological as well as pharmacological tests to identify therapeutically active compounds for preparing modern medicines[2]. But the concentration of the compounds present in these plant parts varies with the seasons and extraction methods[3].

Wrightia 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. They are used in Siddha medicine for the treatment of psoriasis. The major constituents such as indigotin, indirubin, tryptanthrin, isatin, anthranilate and rutin are isolated from *W. tinctoria* leaves. They also contain β-amyrin, lupeol, β-sitosterol and ursolic acid[4]. 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 thermogenic, carminative, anthelmintic, depurative and aphrodisiac properties[5].

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 solubility, safety, ease of working, grade and purity should be considered while selecting the solvent for extracting the plant materials[6]. The phytochemistry or plant chemistry is one of the recent subjects developed as a distinct discipline, which lies in between organic

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chemistry and plant biochemistry[7]. The role of secondary metabolites is previously underestimated since they do not get much involved into the plants primary metabolism. The varied roles of these groups of compounds are realized in recent years[8]. The chemical profile of the plants can be analyzed using certain qualitative chemical tests[9]. Though there are a variety of laboratory methods to measure the antimicrobial action of plant extracts, the most basic and known are the disk-diffusion method and broth or agar dilution method[10]. This study has emphasized to record the pharmacological studies of the traditional medicinal plant *W. tinctoria*. Here, *W. tinctoria* leaf extracts were prepared using several solvents, succeeded by identifying the chemical constituents and determining the antibacterial activity with the aim of finding its suitability for application on wound dressings.

Materials and Methods

Selection of plant

Wrightia tinctoria was selected for the study because of its medicinal properties, ease and availability. The plant was used in the treatment of skin diseases and was also known to possess various pharmacological properties needed for wound healing. Among the various aerial parts such as stems, leaves, petioles, flowers, fruits, seeds and barks, leaves were selected for the study.

Collection and identification of leaves

Fresh leaves of *Wrightia tinctoria* along with stem and flower buds were collected during the flowering season from the agricultural land near Chittode in Erode district for getting authentication. The taxonomic identity of the plant was confirmed as *Wrightia tinctoria* R. Br. belonging to family Apocynaceae by Botanical Survey of India, Tamil Nadu Agricultural University Campus (TNAU), Coimbatore, Tamil Nadu, India (BSI/SRC/5/23/2017/ Tech./3413, Dated 09-03-2017).

Preparation of leaf powder

The collected fresh leaves of *Wrightia 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 completely and stored.

Physicochemical investigation

The following ash and extractive values were determined to evaluate the purity as well as the quality of the *Wrightia tinctoria* leaf powder formulated from the procedure given in several researches[11-13].

Total ash

Wrightia tinctoria leaf powder (about 2 g) was heated up in a silica dish not above 450°C. The process was continued until the drug became white and this indicated that the drug was free from carbon. The residue was allowed to cool and then weighed.

$$\text{Total ash value (\% w/w)} = \frac{\text{Weight of residual ash}}{\text{Weight of original sample}} \times 100$$

Acid insoluble ash

The prepared total ash was initially boiled along with dilute HCl (about 25 mL) for about 5 min. All the insoluble materials were filtered and allowed for washing with hot water. They were heated and then weighed.

$$\text{Acid insoluble ash (\% w/w)} = \frac{\text{Weight of acid insoluble ash}}{\text{Weight of original sample}} \times 100$$

Water soluble ash

The prepared total ash was initially boiled along with water (about 25 mL) for about 5 min. All the insoluble materials were filtered and allowed for washing with hot water. They were heated and then weighed.

$$\text{Water soluble ash (\% w/w)} = \frac{\text{Weight of total ash} - \text{Weight of water soluble ash}}{\text{Weight of original sample}} \times 100$$

Sulphated ash

A platinum crucible was taken and subjected to heat for about 30 min until redness condition. Then it was admitted to cool followed by subsequent weighing. *Wrightia tinctoria* leaf powder (about 1 g) was taken in it followed by addition of 2 mL of dilute H_2SO_4. They were heated to 600°C until the disappearance of all black coloured particles and cooled. Then one or two drops of dilute H_2SO_4 was added followed by the repetition of the same process and weighed.

$$\text{Sulphated ash (\% w/w)} = \frac{\text{Weight of sulphated ash}}{\text{Weight of original sample}} \times 100$$

Water soluble extractive

Wrightia tinctoria leaf powder (about 5 g) was taken in a closed flask in which about 100 mL of chloroform was added. The mixture was frequently shaken for six hours and kept free for the next 18 h. They were then followed by subsequent filtering, evaporating (about 25 mL) and drying at a temperature of 105°C. The weight was calculated to estimate the percentage of water soluble extractive value.

Alcohol soluble extractive

Wrightia tinctoria leaf powder (about 5 g) was taken in a closed flask in which about 100 mL of ethanol (95%) was added. The mixture was frequently shaken for six hours and kept free for the next 18 h. They were then followed by subsequent filtering, evaporating (about 25 mL) and drying at a temperature of 105°C. The weight was calculated to estimate the percentage of alcohol soluble extractive value.

Loss on drying/ Moisture content

Wrightia tinctoria leaf powder was allowed to dry at temperature of 105°C for about 5 h, cooled and again weighed. The procedure was repeated for every one hour and the difference between the weights was calculated to find out the percentage of moisture content.

$$\text{Loss on drying (\%)} = \frac{\text{Loss in weight}}{\text{Weight of original sample}} \times 100$$

Selection of solvents

The solvents help to penetrate the tissues of the leaves and to dissolve its active principles. Petroleum ether, ethyl acetate, ethanol and aqueous solvents were selected for the study.

Extraction method

Continuous hot extraction (Soxhlet extraction) method was selected to separate the medically active portions and soluble plant metabolites of the plant leaves.

Extraction procedure

The extraction of *Wrightia tinctoria* leaf powder (about 40 g) was executed with the help of Soxhlet extractor based on continuous hot extraction method using different solvents of varying polarity (petroleum ether, ethyl acetate, ethanol and water). The solvent from each extraction was distilled off after it becomes colourless, concentrated, evaporated and stored at 4°C using airtight bottles and used for further phytochemical analysis and antibacterial studies.

Qualitative phytochemical screening

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. The extracts obtained were screened using different chemical tests for identification of various phytoconstituents. The extracts were tested for the existence of several phytochemicals as per the methods given by Harborne[7].

Quantitative phytochemical screening

The extracts were quantitatively estimated for determining the total phenolics, tannins, flavonoid content, alkaloid and saponin under the standard test method by Harborne[7].

Yield of the extract

The yield for each solvent extract was calculated in terms of percentage (%). The yield of the extracts was calculated by using the standard formula in which the total mass of the extract is divided by the total mass of the sample multiplied by 100.

Selection of fabric

Viscose spunlace nonwoven was utilized for the study and it was obtained from SITRA, Coimbatore. Viscose was a regenerated cellulosic fiber with a wide spectrum of properties suitable for wound dressings. It was highly absorbent, soft and comfortable and used in most wound dressing materials. The fabric sample was coated with 5% of different solvent extracts of *Wrightia tinctoria* leaves using dip dry method.

Antibacterial activity

The antibacterial activity of the coated fabric samples was tested by disc diffusion method.

Bacterial strains used

The following standard gram negative and gram positive strains commonly found in wounds were utilized.

Escherichia coli and *Pseudomonas aeruginosa* [Gram negative]

Staphylococcus aureus and *Streptococcus Pyogenes* [Gram positive]

The above bacterial strains were obtained from the Department of Pharmacognosy, Nandha College of Pharmacy, Erode.

Preparation of agar

The mixture was prepared using beef infusion (300 g), acid hydrolysate of casein (17.5 g), starch (1.5 g) and

agar (17 g) in distilled water (1 litre). It was then warmed and agar was allowed to get dissolved, followed by sterilization. The procedure was performed using an autoclave for 15 min at 121°C and 15 lbs pressure. The prepared medium was taken in the Petri dishes in sterilized condition.

Antibacterial activity

In this study, disk diffusion method was employed to analyze the antibacterial action of the plant extracts. The extracts dissolved in dimethyl formamide (DMF) (100 µg/mL) was used for the study. The sterilized viscose non-woven fabric in 1 cm diameter was impregnated with prepared solvent extracts using dip dry method. The sterile swab was taken and dipped into the sterilized Petri plates followed by removing the excess inoculum. Streaking was done three times on the surface by rotating the plate (at 60°). They were then subjected to dry for another few minutes in closed form. The different solvent extract coated fabrics were placed in the inoculums with equal distance apart and the non-treated fabric was served as control. The Petri plates were kept in an incubator at about 37°C within half an hour. After the incubation period of 48 h, the zone was determined and expressed in mm.

Statistical analysis

All measurements were made in triplicate. The values were represented as mean ± standard deviation (SD) for each set of experiments. The data obtained in the present study were statistically validated using Microsoft Excel 2019 software package.

Results and Discussion

Extractive and ash values of leaves of *Wrightia tinctoria*

Table 1 showed the extractive and ash values of leaves of *W. tinctoria*. The water soluble ash value was higher than the acid insoluble ash value in the leaf extracts of *W. tinctoria.* Similar findings for the same leaf extract have been reported by Mahadevan *et al.*[14]. In comparison with the value of alcohol soluble extractive, the value of water soluble extractive was found to be higher in *W. tinctoria* which showed that the constituents of the drug were highly soluble and easily extracted in water when compared to that of alcohol[11]. Loss on drying (moisture content) was an important factor that plays a major role in the deterioration of the drug. It was a measure that determines the stability of the drug. The moisture content of the drug was found below 5.94±0.18%.

Table 1 — Extractive and ash values of leaves of *Wrightia tinctoria*

Parameters	Values % (w/w) Mean ± SD
Total ash	11.3±0.33
Acid insoluble ash	0.12±0.08
Water soluble ash	6.46±0.03
Sulphated ash	0.17±0.07
Water soluble extractive	18.8±0.34
Alcohol soluble extractive	6.6±0.23
Loss on Drying/Moisture Content	5.94±0.18

Values are expressed as mean ± SD, Number of replicates (n) = 3

Yield of the plant extracts

The percentage of extraction yield of *W. tinctoria* is given in Table 2. It was observed that aqueous extract produced maximum yield of 17.9% whereas the yield of other solvent extracts (ethanol, petroleum ether and ethyl acetate) were 8.6%, 3.5% and 1.9% respectively.

Phytochemical analysis

Table 3 & 4 showed the results of phytochemicals in the leaf extracts of *W. tinctoria*. The screening exhibited the absence of majority of phytochemicals except phytosterols and sterols in petroleum ether *W.tinctoria* leaf extract. Alkaloids, glycosides, flavonoids, polyphenols and triterpenoids were present in the ethyl acetate extracts of *W. tinctoria.*

Table 2 — Residue of the plant extracts

Sample	Residue (%)
Petroleum ether (PE)	3.5
Ethyl Acetate (EA)	1.9
Ethanol (EL)	8.6
Aqueous (AQ)	17.9

Table 3 — Qualitative phytochemical analysis of different solvent leaf extracts of *Wrightia tinctoria*

Plant constituents	PE	EA	EL	AQ
Test for Alkaloids	-	+	+	-
Test for Glycosides	-	+	+	-
Test for Carbohydrates	-	-	-	+
Test for Phytosterols	+	-	-	-
Test for Steroids	+	-	-	-
Test for Flavonoids	-	+	+	-
Test for Saponins	-	-	+	+
Test for Polyphenols	-	+	+	-
Test for Tannins	-	-	+	+
Test for Proteins and Amino Acids	-	-	-	+
Test for Terpenoids	-	-	-	-
Test for Triterpenoids	-	+	+	-
Test for Fixed Oils and Fats	-	-	-	-

(+) indicates presence of constituents; (-) indicates absence of constituents

PE- Petroleum Ether; EA- Ethyl Acetate; EL- Ethanol; AQ- Aqueous

The ethanol extract showed positive test for alkaloids, glycosides, flavanoids, saponins, polyphenols, tannins and triterpenoids. In aqueous extract, chemical constituents such as carbohydrates, saponins, tannins, proteins and amino acids were present. Terpenoids, fixed oils and fats were found to be absent in all the extracts. Ethanol leaf extract was selected for screening the phytochemicals quantitatively because of the presence of more secondary metabolites viz., alkaloids, glycosides, flavonoids, saponins, polyphenols, tannins and triterpenoids which were responsible for the antimicrobial activity needed for wound healing. The results of quantitative estimation of secondary metabolites existing in the ethanol leaf extract of *W. tinctoria* were summarized in Table 4.

Out of the phytochemicals present in the ethanol extract, alkaloids were the compounds known to have better antimicrobial activity than any other chemical components existing in the plants and they perform a salient part in drug development because of the enormous medicinal properties present in them. Flavonoids present were responsible for enormous medicinal properties such as anti-oxidant, anti-inflammatory and anti-cancer properties[15]. Therapeutic activities were further identified by the presence of saponins that serve as anti-oxidant and anti-inflammatory agents[22]. According to Alabri *et al*[16]., tannins were also considered as one of the primary antioxidants and it was detected in the ethanol extract. More chromogenic reaction was found in the ethanol extract which may be one of the reasons for the presence of these phytochemicals[17]. The high polarity of the solvents may be other reason for the presence of active phytochemical constituents[18]. Senthil *et al.*[19] reported that wound healing process was due to the antioxidant and antimicrobial activities present in the plant extracts.

Table 4 — Quantitative estimation of phytoconstituents in the ethanol extract

Phytochemicals	Quantity mg/g
Alkaloids	4.61 ± 0.31
Flavonoids	15.39 ± 0.27
Phenolics	23.34 ± 0.29
Tannins	7.61 ± 0.18
Saponins	11.24 ± 0.47

Values are expressed as mean ± SD, Number of replicates (n) = 3

Antibacterial activity

The fabrics coated with different extracts were evaluated for their antibacterial potential against four microorganisms using disc diffusion method (Table 5). Out of these, fabric coated with ethanol extract had better antibacterial activity against all bacterial strains. It showed maximum inhibition zone (1.0±0.15 mm to 1.7±0.10 mm) against the bacterial strains followed by the ethyl acetate, petroleum ether and aqueous extract coated fabrics. Flavonoids present in ethanol and ethyl acetate extracts might be the reason for antibacterial activity as reported in a previous study[20]. The maximum inhibitory activity against the growth of all tested gram positive and gram negative bacterial strains was recorded in the ethanol extract coated fabric. Similar results have been reported in the leaf extracts of *W. tinctoria* by Ravi Shankar *et al.*[21]. In the present study, fabric coated with ethanol extract showed better activity due to the presence of active phytochemicals as reported in a study by Poojary *et al.*[22]. Aqueous extract coated fabric was found to have minimum zone of inhibition in the range of 0.1-0.2 mm against four bacterial strains. It was noted that the antibacterial activity has been evaluated in the crude extracts but not on fabrics in all the reported studies. The antibacterial activity of all extract coated fabrics was marked to be better against the chosen gram positive strains. Similar kind of result has also been reported in the ethanol and ethyl acetate extracts of *Artocarpus camansi* leaf extracts[23]. The results proved the remarkable efficiency of the *W. tinctoria* leaf extracts for medicinal applications particularly in developing wound dressings.

Table 5 — Antibacterial activity on coated fabrics

Coated fabrics (with solvent extracts)	Zone of inhibition* (mm) (Activity Index)			
	Gram positive		Gram negative	
	S. aureus	*S. pyogenes*	*E. coli*	*P. aeruginosa*
Petroleum ether	0.3±0.05	0.2±0.03	0.1±0.01	0.2±0.02
Ethyl acetate	0.9±0.10	0.7±0.05	0.5±0.05	0.8±0.01
Ethanol	1.4±0.06	1.7±0.10	1.2±0.03	1.0±0.15
Aqueous	0.2±0.02	0.2±0.01	0.1±0.01	0.1±0.01

Values are expressed as mean ± SD, Number of replicates (n) = 3

Conclusion

The study exhibited the biological and pharmacological activities of different solvent extracts of *Wrightia tinctoria* leaves. The results of phytochemical investigation revealed the presence of more secondary metabolites in *W. tinctoria* which indicates the pharmacological and medicinal value of the plant. The results of this study clearly indicate that the fabric coated with ethanol extract of *W. tinctoria* leaves contains the major chemical components and showed better antibacterial activity. Also it was suggested to coat the viscose nonwoven fabric using other coating techniques. This plant can be successfully exploited for application on wound dressings due to the presence of more pharmacological activities and it was suggested to isolate the bioactive compounds responsible for the drug development. Further studies were required to improve the antibacterial activity by adopting different coating techniques for developing ideal wound dressing materials.

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Conflict of Interest

The authors of the present research work affirm that there was no conflict of interest.

Authors' Contributions

NCM- literature survey, research design, manuscript writing, experimental work; SLM- research design, manuscript editing, experimental work; GY- literature survey, experimental work; RD- research design, experimental work.

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