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Pharmacognostical, Phytochemical and Antibacterial Evaluation of *Berberis Tinctoria* Lesch. (Stem Wood and Stem Bark).

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ABSTRACT

Pharmacognostical, Phytochemical and Antibacterial studies of *Berberis tinctoria* Lesch. (stem wood and stem bark).The present study has been undertaken to evolve the Pharmacognostical, Phytochemical and Antibacterial standard. In Pharmacognostical study determination of macroscopical and microscopical characters were carried out like colour, taste, odour, ash value extractive value and section study. In Phytochemical study Extraction procedure, Fluorescence analysis, chemical analysis, berberine isolation, TLC, and HPTLC were carried out. In Microbiological study the antibacterial activity of methanolic extracts was carried by cylinder-plate and serial dilution method. Pharmacognostical studies such as ash value and extractive value were carried out to confirm the identity of plant and to ascertain the quality and purity of drug. Microscopical study (stem wood and stem bark) showed the presence of different types of tissues. Phytochemical study such as Fluorescence analysis shows fluorescence compound in the extract, chemical analysis, shows the presence of alkaloid (berberine). Microbiological study such as antibacterial study showed the prominent antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* in comparison to standard (Ampicillin trihydrate) Pharmacognostical studies were carried out to confirm the identity and to ascertain the quality and purity of the drug. Phytochemical studies were carried out to confirm the presence, nature and amount of active constituents. The extract of (stem wood and stem bark) evaluated for antibacterial activity and showed prominent antibacterial activity.

Keywords: *Berberis tinctoria* Lesch. , Berberine, Stem wood, Stem bark, Ampicillin trihydrate.

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INTRODUCTION

Berberis tinctoria Lesch. (Family Berberidaceae), is found in Nilgiri and Pulney Hills of the Western Ghats at an altitude of about 6000ft [1] . It has been reported that the alcoholic extracts of the (stem wood and stem bark) of the plant was found to have cardiovascular and diuretic activity [2] and the methanolic extract of the root has significant antibacterial activity [3]. Antimicrobial activity of the aqueous root paste has been reported by the native and tribes of Nilgiris [4]. The plant is reported to contain alkaloids, like berberine, berbamine, jatrorrhizine, umbellatine, neprotine and palmatine [5]. We report herein the isolation characterization and quantitative estimation of alkaloid berberine and also inhibitory effect of the extracts in various strains of certain micro-organisms.

MATERIAL AND METHODS

Collection and Treatment

The plant *Berberis tinctoria* Lesch. is found in various parts of Nilgiris, such as Ootacamund, Pykara and Kotagiri. For my work, the plant was collected from Doddabetta region of Ootacamund by peeling the stem in the month of June and was identified, confirmed and authenticated by botanist Dr. Suresh Baburaj, Botanical Survey of Medicinal Plant, Central Council for Research in Homoeopathy, Government Arts College Campus, Ootacamund. The stem wood and stem bark were washed with running tap water to remove adhering unwanted materials. The stem wood and stem barks were cut into small pieces with stainless steel knife and were dried at temperature not exceeding 40 °C and powdered. The powdered drugs were extracted by methanol by soxhlet apparatus for six hours[6] and the fluorescence character of the extract were studied both in day light and UV light[7]. The observation is shown in (Table-1)

Table – 1: Fluorescence Analysis of Methanolic Extracts of Stem wood and stem bark

Types of extracts	Day light	UV light	
		Long UV (365nm)	Short UV (254nm)
Methanolic extract (stem wood)	Brownish yellow	Green	Yellow
Methanolic extract (stem bark)	Brownish yellow	Green	Yellow

Determination of Ash Values and Extractive Values [8, 9]

Total ash, acid insoluble ash, water soluble ash and sulphated ash for the stem wood and stem bark were determined (Table - 2)

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Table – 2: Ash Values of Different Parts of *Berberis tinctoria* Lesch.

Types of ash values	Ash values (%w/w)	
	Stem wood	Stem bark
Total ash	2.68	4.96
Acid insoluble ash	1.37	2.52
Water soluble ash	0.79	1.75
Sulphated ash	3.36	6.72

Extractive values such as alcohol soluble, water soluble and ether soluble for the stem wood and stem bark were also determined (Table - 3).

Table - 3 Extractive Values of Different Parts of *Berberis tinctoria* Lesch.

Types of extract	Extractive values (%w/w)	
	Stem wood	Stem bark
Alcohol soluble extractive	8.62	8.63
Water soluble extractive	4.57	4.52
Ether soluble extractive	0.35	0.27

Isolation and Characterisation of Berberine

Berberines were isolated from stem wood and stem bark by following the standard procedure[9] and were characterised by the study of physical characterstics[10], chemical test6, spectral[10] and Thin Layer Chromatography (TLC) [11] analysis (Table - 4)

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Table – 4: Characterisation of Isolated Berberine

Types of studies followed	Parameters studied	Observations	
		Berberine (Stem wood)	**Berberine (Stem bark)**
Physical Characteristic	Colour	Yellow crystalline powder	Yellow crystalline powder
	odour	Odourless	Odourless
	Taste	Very bitter	Very bitter
	Solubility	Ethanol, Methanol and hot water	Ethanol, Methanol and hot water
	Melting point (^{0}C)	145 (Uncorrected)	145 (Uncorrected)
Chemical test	Tests for alkaloids	Positive response	Positive response
Spectral analysis	UV (λ max nm)	344.80	346.00
	IR (cm^{-1})	1598.1 1507.4 1363.0 1275.0 1230.4 1035.3	1598.7 1507.7 1364.3 1277.4 1230.0 1035.7
TLC analysis [Mobile phase-: n-propanol-formic acid-water (90: 1: 9)]	Number of sports Colour of the sport (Day and UV light)	One spot Yellow	One spot Yellow
	R_f value	0.40	0.42

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Antibacterial Studies

Both gram - positive and gram - negative strains (*Staphylococcus aureus* and *Escherichia coli* respectively*)* have been used for the studies. Nutrient agar media was used for the growth of bacteria for cylinder - plate method and nutrient broth media for serial dilution method[12]. The methanolic extracts (stem wood and stem bark) and the standard drug (Ampicillin trihydrate) was dissolved in dimethysulphoxide (DMSO) at the concentration of 20 mg/ml and 1 mg/ml respectively. The results are tabulated (Table - 5 and 6).

Table – 5: Antibacterial Study of Methanolic Extracts of *Berberis tinctoria* Lesch. by Cylinder - Plate Method

Name of bacteria	Mean diameter of zone of inhibition (cm)		
	Stem wood 20 mg/ml	Stem bark 20 mg/ml	Standard drug (Ampicillin trihydrate 1mg/ml)
Staphylococcus aureus	2.57	2.87	4.25
Escherichia coli	2.17	2.27	4.10

Table – 6: Antibacterial study of Methanolic Extracts of *Berberis tinctoria* Lesch. by Serial Dilution Method

Name of bacteria	MIC (µg/ml)			
	Stem wood extract	Stem bark extract	Standard drug (Ampicillin trihydrate)	Solvent (DMSO)
Staphylococcus aureus	500	250	50	-
Escherichia coli	2000	-	50	-

Note (-) no inhibition at the concentration of 2000 µg/ml

Quantitative Estimation of Berberine of Stem wood and stem bark of *Berberis tinctoria* Lesch. by HPTLC technique

Methanolic extract of stem wood and stem bark were subjected to HPTLC analysis for the quantitative estimation of berberine. Sample solution of the extracts was prepared at the concentration of 1% w/v and the standard solution of berberine was prepared at the same concentration in methanol. Pre-coated plates (Silica gel 60 F254 Merck) were used as the stationary phase. The sample solution and the standard berberine solution were applied as a thin band of 6 mm width on the plate by using Camag Linomat IV n-Propanol-formic acid- water (90:1:9) was used as the mobile phase[11]. The applied plates after drying were developed in previously saturated Camag Twin Trough Camber. After proper development, the plates were removed from the chamber and air dried. Densitometric evaluations of the developed plates were carried out by using Camag TLC scanner 3 stationed with Camag Cats software programme for integration at 254 nm. Integration Calibration Spectra was recorded for the samples and the total area included in the peak corresponding to berberine was observed. The quantitative estimation of berberine of the extracts was carried out from

the total area included in the peak corresponding to berberine with reference to the standard berberine (Table - 7).

Table - 7: Quantitative Estimation of Berberine of Stem wood and stem bark Extracts *of Berberis tinctoria Lesch.* by HPTLC Technique.

Types of samples	Quantity of sample applied (µg)	Rf value	Total area included in the peak	Percentage of berberine (% w/w)
Methanolic extract (Stem wood)	10	0.40	28466.4	46.36
Methanolic extract (Stem bark)	10	0.42	32775.0	53.19

RESULTS AND DISCUSSION

The methanolic extraction was done by taking the stem wood and stem bark of the *Berberis tinctoria* Lesch. and it was found that the methanolic extracts have high extractive values when compare to other extractive value (water soluble, and Ether soluble extractive value).The fluorescence analysis of the extracts showed the presence of fluorescent compounds in all the extract. The ash values and extractive value of stem wood and stem bark of the plant were determined. From the isolation of the berberine, from the stem wood and stem bark it was found that the bark contains more amount of berberine than the wood portion. The isolated berberines were characterized by the study of Physical characteristics, Chemical test, Spectral and TLC analysis. From the quantitative estimation of berberine of the stem wood and stem bark extract by HPTLC technique and it was also confirmed that the more amount of berberine is present in the methanolic extract of stem bark than stem wood of *Berberis tinctoria* Lesch. Antibacterial activity of the compound was determined by Cylinder-plate method and serial dilution method. The result represents that the antibacterial activity is prominent. The methanolic extract is more active against *Staphylococcus aureus* in comparison to *Escherichia coli*.

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