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Phytochemical Analysis and Antimicrobial Activity of *Vallaris solanacea* Leaves Extract

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ABSTRACT

The Apocynaceae family plants have a variety of traditional uses, and it has practiced for a long time. *Vallaris solanecea* belongs to this family. This species is famous as an ornamental plant garden in Southeast Asia. The objective of this research is to estimate the antibiotic activity and the MIC in *Vallaris solanecea* specimens of chosen bacteria. The antibacterial properties in the samples were assessed against the bacterial pathogens - two gram-negative *Salmonella typhi* and *Escherichia coli*, also in terms of gram-positive *Enterococcus faecalis* activity. In this research of antimicrobial operation by the agar, well diffusion technique, petroleum-ether, chloroform and ethanol, and methanol and aqueous specimens were used. The antibacterial activity of samples was assessed around the wells to verify the microbial resistance. Results indicate that all the extracts were effective against tested organisms in comparison to standarddrugs. Petroleum-ether and methanol extract exhibited a relatively higher zone of inhibition against *S. typhi*, *E. coli, E. faecalis*. Chloroform and ethanol extracts showed a satisfactory zone of inhibition against all the pathogens as compared to standard drug.

Keywords: *Vallaris solanacea*, Antibiotic, Anti-bacteria, Anti-Microbial, Gram-Positive

INTRODUCTION

The knowledge about medicinal plants used for the treatment of diseases was unknown to people in the past. They came to know only by the experimental procedure or by trial and error method. During the iatrochemistry (16th century), people became aware of the source treatment and prophylaxis about the plants [1]. The family of Apocynaceae consists of 250 genera and 2000 species [2] and is enlarging from two to five subfamilies [3]. These are mainly tropical trees, shrubs, and vines. The main characteristic property of this family is, all the species produce milky sap; leaves are simple, opposite, or whorled; flowers are large, colourful, and slightly fragrant with five contorted lobes; and fruits are in paired [4]. This plant species is used mainly for the treatment of gastrointestinal ailments, fever, malaria, pain, and diabetes [2]. It also has anticancer properties and was checked against six cancer cell lines [4]. *Vallaris solanaceae* [Roth] Kuntz belongs to Apocynaceae is a 10m twist shrub climbing with dirty white-grey bark and having a flowering branchlets which are white and creamy, flavourful with an oblique and densely pubescent on both the surfaces of the leaves (Figure 1) and mostly seen in China, India, Pakistan, Sri Lanka, Cambodia, Myanmar, Thailand, Vietnam, and Indonesia. In India, it was found in the Malnad region of Karnataka, India. It has a very good medicinal value for skin disease treatment, wound healing, and many more [5,6].

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The stem of the plant has an antiulcer antioxidant activity, which was expressed in Wistar Albino Rat [7]. The milky latex of the plant has many applications that are used for the treatment for ringworm, skin infections. The oils from the bark have anticancer, antimicrobial, analgesic, anti-inflammatory, anti-diarrheal, and

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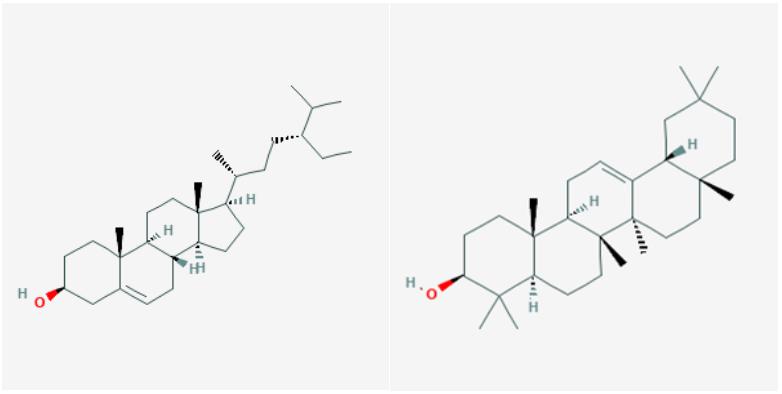
cardiotonic properties [5].

Figure 1: Flowers and leaves of *Vallaris solanacea* [5]

Phytochemistry

Cardiac glycosides: The seed of *V. solanaceae* has many chemical compounds like glycosides of vallaroside, solanoside, vallarosolanoside, 16-diacetyl-16-anhydro-acoschimperoside P, mono-O-acetyl-acoschimperoside P, mono-O-acetyl-vallaroside and mono-O-acetyl- solanoside [5]. From this, O-acetyl-solanoside was isolated, and on further analysis shows the presence of β- sitosterol, β-amyrin (Figure 2), ursolic acid, vallaroside, solanoside, vallarosolanoside and acoschimperoside [8]. Recent studies show a new glycoside called cardenolide have been identified and compounds have been isolated named as 3β-O-[a-acofriosyl]-16-anhydrogitoxigenin, along with benzyl 2-O-β-apiofuranosyl-[1/2]-β-D-glucopyranosyl-2,6-dihydroxy-benzoate,having a molecular formulae of $C_{32}H_{46}O_9$, $C_{30}H_{44}O_8$ and $C_{25}H_{30}O_{13}$ with their molecular weight of 597.3, 571.0 and 561.2 respectively [5].

Chemical structure of *V. solanaceae* bioactive compounds



[A] [B]

Figure 2: [A] shows β sitosterol, and [B] shows β amyrin [9,10]

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Other compounds

The root bark of *V. solanaceae* was studied and it consists of eight fatty acids with arachidonic acid [42%], palmitic acid [38%], and capric acid [15%] as the main components [8-11].

MATERIALS AND METHODS

Collection of leaves sample

The fresh leaves of *Vallaris solanacea* were collected from the Konanduru village, present in Thirthahalli taluk, Shimoga district, Karnataka, India. The plant was identified by the botanist from the Department of Applied Botany, Maharani Science College, Mysore, Karnataka. The leaves were brought into the laboratory and washed with tap water removing the dust particles. The cleaned plant leaves were shade dried for seven days at 37^{o}C room temperature. About 50grm of shade dried leaves were powder with the help of mortar and pestle and subjected to the sequential Soxhlet extraction by using petroleum ether, chloroform, methanol, and ethanol, respectively. The extracts were kept at 4^{o}C to avoid contamination until further use.

Preparation of *V. solanacea* leaves extract

Freshly prepared leaves dry powder was taken, and sequentially extracted by using Soxhlet extractor with optimal conditions. Fifty grams of leaves dry powder was used with 250 ml of different solvents such as petroleum ether, chloroform, methanol, ethanol, and aqueous hot and cold. The operating temperature for this experiment varied from 30^{o}C to 40^{o}C. The complete sample was recovered fully, and it was collected in a thimble. The obtained leaves extraction was collected and stored at room temperature for further use. The extraction is done for all the six solvents, and their yields were noted.

Phytochemical analysis

Qualitative analysis was done using the *V. solanacea* plant extracted by using different solvents in Soxhlet extractor. The test name, phytochemical, and the reference is mentioned in the Table 1.

Table 1: Phytochemical analysis of *V. solanacea leaf* extract

Sl.NO.	PHYTOCHEMICAL TETS	TEST NAME	REFERENCES
1.	ALKALOIDS	WAGNER'S TEST	[12]
2.	CARBOHYDRATES	MOLISH TEST	[13]
3.	STEROIDS	SALKOWSKI TEST	[14]
4.	TERPENOIDS	SALKOWSKI TEST	[14]
5.	FLAVONOIDS	ALKALINE REAGENT TEST	[14]
6.	REDUCING SUGAR	BENEDICT'S TEST	[14]
7.	AMINO ACIDS	NINHYDRINTEST	[14]
8.	GLYCOSIDES	BORNTRAGER'S TEST	[14]
9.	PROTEINS	BIURET TEST	[14]
10.	PHENOLS	$FeCl_3$ TEST	[14]

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11.	TANNINS	$FeCl_3$ TEST	[14]
12.	SAPONINS	FOAM TEST	[15]

Anti-Microbial activity

Microbial strains

Microbial strains were collected from the JSS Medical College, Mysore, Karnataka. The antimicrobial activity of plant extracts was carried out against four microbial strains using the agar well diffusion method. Both gram-positive and gram-negative bacteria were taken for testing. Gram-positive *Staphylococcus aureus, Enterococcus faecalis,* and Gram-negative, *Salmonella typhi,* and *Escherichia coli* were taken and were performed against 24-hour culture. Gentamicin was used as an antibiotic drug to compare antibacterial activity, and it was tested against the bacterial strains at three concentrations of 30, 40, and 50µg/ml. After the 24 hours of incubation, the zone was noted [6].

Inoculum

The inoculating loop was used to pick three to four colonies and suspended in 5 ml broth and incubated for 24 hours at 37^{o}C. The broth culture turbidity was then balanced to match 0.5 MacFarland's standards [16].

Cork Borer method

In these methods, 25 ml of nutrient agar was poured into sterile Petri dishes. The agar was punched out at equally spaced positions with a sterile bore [back hole] to make five holes after congealing. The test sample was solution was filled into the holes with 30, 40, and 50µl while the fifth was filled with antibiotic Gentamicin. The plates were then left for 2 hours at room temperature [to promote diffusion over microbial growth] and incubated for 24 hours in an incubator at 37°C. The antibacterial activity was evaluated using a ruler to measure the diameter of the inhibition zone [17].

Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration is defined as the lowest concentration of test samples resulting in complete inhibition of visible growth. A micro dilution method was used to determine the MIC of all extracts. The respective clinical strain was spread on the medium. The wells were created using a sterilized cork borer in stainless steel under aseptic conditions. The extracts in different concentrations viz. 30, 40, and 50µg loaded in the corresponding wells. The standard drug Gentamicin (40µg in 100µl) was used as standard drugs to compare antibacterial activities. The inhibition zone was compared with the standard drug after 24 hours of incubation at 37^{o}C for antibacterial activity [6].

RESULTS

The *V. solanacea* plant leaf sequential extraction extracted by using Soxhlet extractor, yield (ml) was tabulated as below Table 2.

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Sequential solvent extraction from *V. solanaceae* plant leaves

Table 2: Yield of leaves extract in different solvents

SOLVENT USED		FRESH LEAVES YIELD (m)]
Petroleum ether		30
Chloroform		28
Methanol		20
Ethanol		13
Aqueous	Hot	20
	Cold	10

Phytochemical analysis report: Standard methods are used for testing the presence or absence of various phytochemical in the solanaceae extracts, that are given in the below Table 3.

Table 3: Phytochemical screening of bioactive compounds in *V. solanacea* plant extract

S. No	Bio-active compounds	Non-polar solvents Petroleum ether	Mini polar solvents chloroform	Polar solvents		Aqueous	
				Methanol	Ethanol	Hot	Cold
1	Alkaloids	-	+	-	-	-	-
2	Carbohydrates	+	+	+	+	+	+
3	Steroids	+	+	+	+	+	+
4	Terpenoids	-	+	-	-	+	-
5	Flavonoids	+	+	+	+	+	+
6	Reducing sugar	+	+	+	+	+	+
8	Amino acids	+	-	+	-	+	-
9	Glycosides	+	+	+	+	+	+
10	Proteins	+	-	+	-	+	-
11	Phenolic and tannins	+	+	+	+	+	+
12	Saponins	+	-	+	-	+	-

The Wagner's test for the test of alkaloids showed a positive presence of reddish-brown precipitate in mini polar chloroform extract and negative in petroleum ether, methanol, and ethanol, aqueous hot and cold solvents. Molish test for carbohydrate also showed a violet ring at the junction in all the

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extracts. Salkowski's test for steroid and terpenoids had a violet/green/reddish/ brown ring color is positive in all the extract for the bioactive compound steroid but showed the negative result in petroleum ether, methanol, ethanol and aqueous cold solvents for the bioactive compound terpenoids. The alkaline reagent test for flavonoids showed a positive result in all extracts where yellow color becomes colorless. Benedict's test for testing reducing sugar showed a green or yellow or red precipitate in all the extract solvent. Ninhydrin test for amino acid showed a positive blue color from the extract's petroleum ether, methanol, and hot aqueous solvents. Keller Kilian test for glycosides formed a reddish-brown color at the junction of two solutions in all the extracts. Biuret test for the presence of protein showed the positive presence of violet/ pink color reference form the extracts of petroleum ether, methanol, and hot aqueous solvents. Ferric chloride test for phenols (5%) and tannins (10%) compounds showed the formation of blue or violet color and blue, green color in all the extracts solvents. Froth test for saponin showed the appearance of foam in petroleum ether, methanol, and hot aqueous solvents.

Antimicrobial activity

The antimicrobial activity of the plant extracts against *E. coli*, *E. faecalis*, *S. typhi* by measuring the diameter of zone of inhibition are shown in the Table 4.

Table 4: Zone of inhibition against *E.coli*, *E. faecalis* and *S.typhi*

Zone of Inhibition (mm) Dilution of plant extract (µg/ml)													
Organism	**Control**	**Methanol**			**Ethanol**			**Chloroform**			**Aqueous**		
		30 µg/ml	40 µg/ml	50 µg/ml	30 µg/ml	40 µg/ml	50 µg/ml	30 µg/ml	40 µg/ml	50 µg/ml	30µg/ml	40 µg/ml	50 µg/ml
E.coli	-	3.5mm	3.5mm	5mm	-	6.2mm	6.2mm	7mm	7.4mm	7mm	-	-	8mm
E.faecalis	-	8mm	6mm	8mm	5mm	5.1mm	5.1mm	8mm	10mm	10mm	6mm	6mm	6mm
S.typhi	-	11mm	14mm	15mm	09mm	12mm	12mm	-	-	14mm	8mm	8mm	8mm

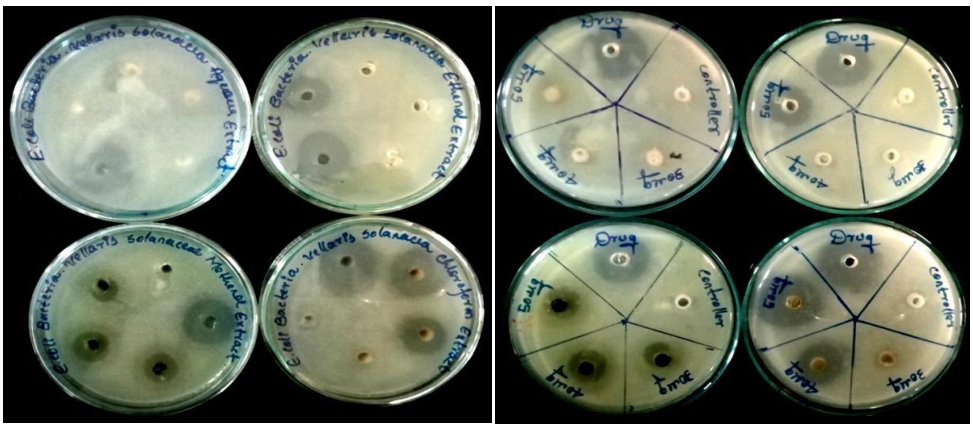

Figure 3: Zone of inhibition of plant extract against *Escherichia coli*

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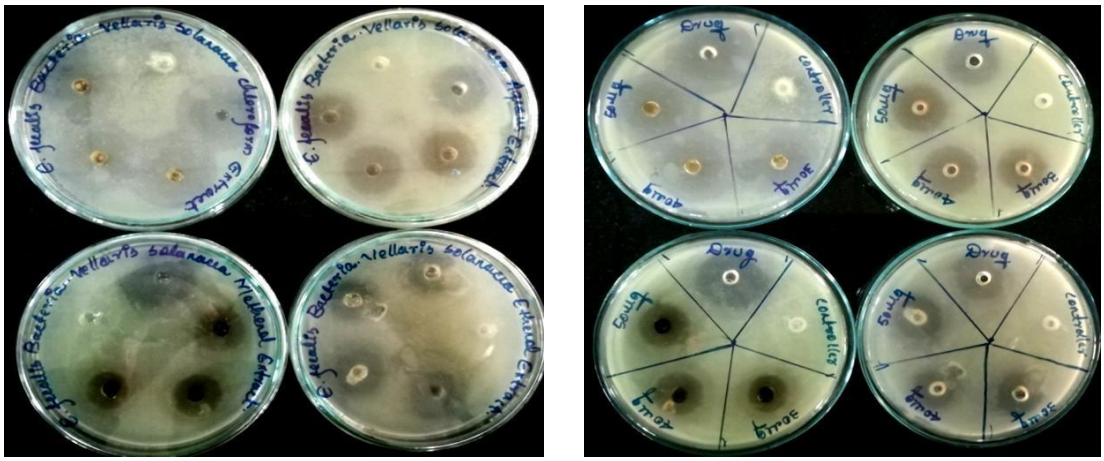

Figure 4: Zone of inhibition of plant extract against *Enterococcus faecalis*

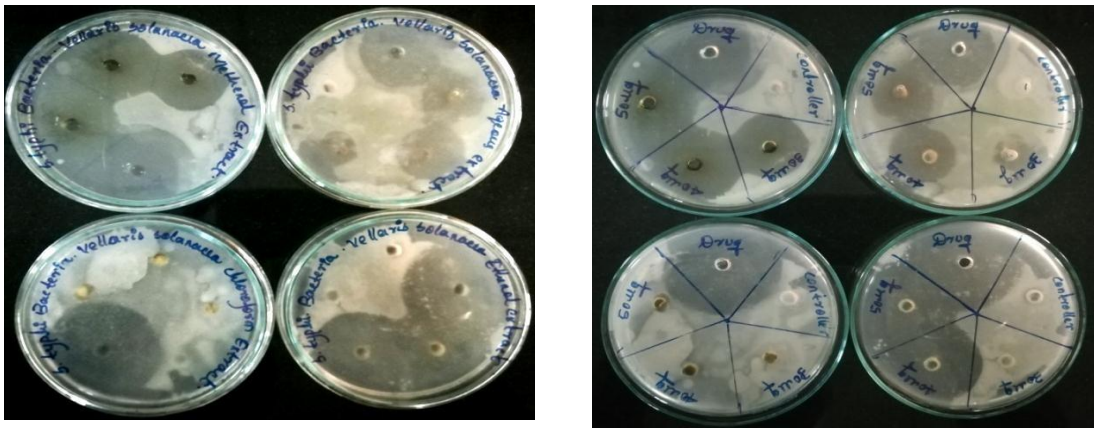

Figure 5: Zone of inhibition of plant extract against *Salmonella typhi*

Antimicrobial Assay

The *Vallaris solanaceae* extracts have shown pronounced in vitro anti-microbial activity potential for all four bacterial species. The extracts have been tested against bacterial strains at three concentrations. The results of the well-diffusion method showed that *S. typhi, E. coli,* and *E. faecalis*, showed a high level of antimicrobial activity in this methanol extract. *E. faecalis, E. coli* with most strong inhibition zones [10 mm, 10 mm] and showed a maximum effect from chloroform extract (Table 4). Antimicrobial activity against *S. typhis, E. faecalis* was high in the ethanol extract.

MIC shows that methanol extract was highly sensitive against *S. typhi, E.coli,* and *E.faecalis*, as a result of minimum inhibitor concentration. During chloroform extracts, *E. faecalis* and *S. typhi* were highly sensitive to *E. coli*. The ethanol extract was stronger than the standard drug for *S. typhi,* followed by *E. coli* and *E. faecalis* (Figure 3-5).

DISCUSSION

Plants have the capability of producing many bioactive compounds. The high concentration of phytochemicals seen in fruits and vegetables leads to the protection against free radicle damage. The phytochemical analysis of the leaves of *Vallaris solanacea* showed both the medicinal and the physiological components of the plant. The extracts from Chloroform, petroleum ether, ethanol, methanol, and distilled water were used to examine the phytochemical analysis. This plant hasanti-cancer property, whereby showing resistance to TRAIL (tumor necrosis factor-related apoptosis-

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inducing ligand) and has overcome in human gastric adenocarcinoma (AGS) cells and cell growth-inhibitory activity towards HeLa and SW480 cells. Extract from stem bark has an inhibitory growth action against gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis)* and gram-negative bacteria (*Salmonella typhi* and *Escherichia coli)* and some fungi (*Candida albicans* and *Aspergillus niger)*. The extracts from leave and stems by using ethanol have analgesic activity in acetic- acid inhibition in mice with two doses of 500mg/kg and 250mg/kg, where 54% and 23%. It also has significantly reduced paw volume in the 1st to 5th hour using the rat paw induced and carrageenin with the highest 3rd hour, which was 29% and 41% for the doses 200mg/kg and 400mg/kg respectively. It had a cardiac glycoside named O-Acetyl-solanoside was found to possess cardiotonic activity in cats and guinea pigs. The toxicity study was done on albino rats from the root bark of *V. solanaceae* for the study of essential oil. Two groups of rats weighing 150mg and 200 mg were orally fed with oil at a dose of 3ml/kg and 5 ml/kg. The ethanol extract of leaves and stem showed a little amount of toxicity of LC50 of 80mg/ml and LC90 of 320mg/ml.The Fehling's and Benedict's test showed positive for all the six samples. Chloroform and aqueous hot extracts were detected with terpenoids. All the samples were shown negative results for Wagner's test except the aqueous cold, indicating the absence of alkaloids. The sample extracts from methanol, petroleum ether, and hot water showed a positive froth foam test, which determines saponin. The petroleum ether and methanol extract detected anthraquinones and phlobatannins. Steroids were detected in all the six samples. Glycosides, phenolics, and tannins were found among all six solvent extracts, including phytochemical flavonoids.The volatile oil was found out in *Vallaris solanacea's* leaf extract from methanol, petroleum ether, and hot distilled water. This shows the therapeutic use and confirmed its ethnomedicinal claims of traditional plants. The above-mentioned results confirm the antimicrobial activity of *V. solanacea* against selected bacterial strains. The inhibitory action of the plant depends on the part of the plant used, its concentration, and the selection of microbes.

In polar solvents like methanol, ethanol, the efficacy of the extracted phytochemicals was shown more than the non- polar solvents like chloroform, petroleum ether. The majority of the plant chemicals were tested in distilled water, ethanol, and methanol. The *Vallaris solanecea* extracts have shown pronounced in vitro anti-microbial activity potential for all three bacterial species. The extracts have been tested against bacterial strains at three concentrations. The results of the well-diffusion method showed that *S. typhi, E.coli*, and *E. faecalis,* showed a high level of antimicrobial activity in this methanol extract. *E. faecalis, E. coli* with most strong inhibition zones [10 mm, 10 mm] showed a maximum effect from chloroform extract. Antimicrobial activity against *S. typhis, E. faecalis* was high in the ethanol extract. In comparison with standard drug, with an inhibition zone of 11 mm**.** MIC shows that methanol extract was highly sensitive against *S. typhi, E. coli,* and *E. faecalis,* as a result of minimum inhibitor concentration. During chloroform extracts, *E. faecalis* and *S. typhi* were highly sensitive to *E. coli*. The ethanol extract was stronger than the standard drug for *S. typhi* followed by *E. coli and E. faecalis*. Further studies are needed in this plant to isolate and characterize the compounds of the bioactive principle to develop a new antibacterial drug.

CONCLUSION

The oldest form of medicine in southern Asia, particularly in India, is Ayurveda and is practiced under Veda and Upanishad. The main principle of this medicinal practice is the application of medicinal plants and their compounds in a methodology specified for spiritual and physical well-being and health. The science explaining the expression of these medicinal properties is secondary metabolites secretion, which is useful for other organisms, and which is not used for plant growth and development. Through the plant-specific, these biochemical compounds act and interact with other biomolecules, and either the form enzymes of the active biological peptides result in the necessary primary or secondary changes. The therapeutic and medicinal properties expressed these changes. The present work has revealed that different components of the plant are found in *Vallaris solanaceous* leaves, such as carbohydrates, flavonoids, saponins, phenols, tannins, glycosides, and

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steroids. Many medicinal therapies treat herbal medicines for their extraordinary effect, although relatively little knowledge of how they work is possible. Herbal extracts have long been used in the Ayurvedic medicine system instead of purified compounds, as many elements with more than one mechanical action are regarded as essential for the necessary holistic therapeutic action.This work can be a valuable information source and provide appropriate standards for establishing the quality of this plant material in the forthcoming future study.

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