



Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd



Document heading

doi: 10.1016/S2222-1808(14)60639-3

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Preliminary phytochemical screening and *in vitro* antibacterial activity of *Bauhinia variegata* Linn. against human pathogens

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PEER REVIEW

Peer reviewer

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Comments

It is a good study in which the author evaluated the antimicrobial and phytochemical properties of the well known traditional plant that is found to have good antimicrobial activity. The result is very interesting and is significant in terms of qualitative authentication of the age old claim. The result and such documentation are very important for natural drug development as well. I strongly recommended for publication in your esteemed journal.

Details on Page 128

ABSTRACT

Objective: To investigate the antimicrobial and phytochemical properties of hydromethanolic extracts of *Bauhinia variegata* Linn. (*B. variegata*) (leaf, stem bark and flower) to justify the traditional claim endowed upon this herbal drug as a rasayana in Ayurveda. This study thus can be further utilized to formulate the natural antioxidant which can be used as a dietary supplement to fight against several diseases such as cancer, ageing, artherosclerosis, etc.

Methods: The study showed that the number of different phytoconstituents present in the plant which makes it remarkable for its use by traditional practitioners. On the another set of experiment, the hydromethanolic extract of *B. variegata* (leaf, stem bark and flower) were evaluated against Gram-positive and Gram-negative by using disk diffusion assay.

Results: Phytochemical screening of all extracts showed the presence of alkaloids, steroids, phenolic compounds, tannins, saponin, carbohydrates, proteins, amino acids and organic acids. The antibacterial activity of all the extracts (leaf, stem bark and flower) of *B. variegata* was determined by agar well diffusion method at four different concentrations i.e., 1000 mg/mL, 750 mg/mL, 500 mg/mL and 250 mg/mL using Gram-positive *Bacillus subtilius*, *Staphylococcus aureus* and *Streptococcus epidermidis* and Gram-negative *Escherichia coli*, *Shigella flexineria*, *Pseudomonas auriginosa* bacteria.

Conclusions: These studies show that hydromethanolic extracts of *B. variegata* (leaf, stem bark and flower) inhibited the growth of microorganism's in dose dependently. *B. variegata* leaf, stem bark and flower extracts have several phytochemical constituents who possess the antimicrobial activity. A tiny amount of data is presented, as the preliminary antimicrobial properties of the *B. variegata* here accessed, under the urgent necessity of new antibiotics in the market and in face of the increased resistance of infectious microorganisms to antimicrobials.

KEYWORDS

Phytochemical constituents, Antibacterial activity, Disc diffusion, Medicinal plants

1. Introduction

Nowadays, an increasing number of infectious agents are becoming more resistant to commercial antimicrobial compounds[1]. The necessity to develop new drugs requires varied strategies, the bioprospection of secondary metabolites produced by medicinal plants is one of them[2,3].

Antimicrobial agents are undeniably one of the most important therapeutic discoveries of the 20th century. However, with the 'antibiotic era' barely five decades old, mankind is now faced with the global problem of emerging resistance in virtually all pathogens[4].

During the last decades, there is an increasing interest to unlock the secrets of ancient herbal remedies. For

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Foundation Project: Supported by DST, New Delhi, (Grant No. DST No. Letter no.SSD/SS/010/2010.

Article history:

Received 12 Dec 2013

Received in revised form 24 Dec, 2nd revised form 29 Dec, 3rd revised form 31 Dec 2013

Accepted 2 Mar 2014

Available online 31 Jul 2014

this purpose, various strategies have been developed *e.g.*, biological screening, isolation as well as clinical trials for a variety of plants[5].

In recent years, many possible sources of natural antibiotics have been in use for several infectious diseases, mostly bacterial and fungal. In view of this, the searches for new anti-microbial agents from medicinal plants are even more urgent in the countries like India where infectious diseases of bacterial origin are not only rampant, but the causative agents are also developing an increasing resistance against many of the commonly used antibiotics[6]. Considering the high costs of the synthetic drugs and their various side effects, the search for alternative products from plants used in folklore medicine is further justified. It is believed that plants which are rich in a wide variety of secondary metabolites belonging to chemical classes such as sterols, alkaloids, glycosides, saponins, flavonoids, tannins, and carbohydrates are generally superior in their antimicrobial activities[7]. For example, quinine (*Cinchona*) and berberin (*Berberis*) are alkaloids obtained from plants which are highly effective against microbes such as *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*)[5]. Therefore, the determination of the compounds responsible for any biological activity would facilitate the selection of the plants for future investigations.

The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials. Continued further exploration of plant-derived antimicrobials is needed today. Further research is necessary to determine the identity of the antibacterial compounds from within these plants and also to determine their full spectrum of efficacy. However, the present study of *in vitro* antimicrobial evaluation of some plants forms a primary platform for further phytochemical and pharmacological studies.

Bauhinia variegata Linn. (Leguminosae) (*B. variegata*), is known as Kanchanar in Hindi, is a medium sized tree abundant in Sub-Himalayan tract extending eastwards to Assam, Eastern, Central and South India[8]. The various parts of the plants *viz.*, leaves, flower buds, flower, stem, stem bark, seeds and roots are used in fever, as tonic, astringent, diarrhoea, dysentery, hemorrhoids, piles, edema, laxative, anthelmintic, antileprotic, in skin diseases, wound healing, antigoutogenic, antitumor, in obesity, stomatitis, antidote for snake poisoning, dyspepsia, flatulence and as carminative[9].

The purpose of this study was to carry out preclinical evaluation of some popular medicinal plant species, *i.e.*, biological and phytochemical screening with particular emphasis on those that seems to have very little scientific information in the areas intended for the investigation. This study facilitated the selection of plants with relatively high level of potency and wide range of biological activities

suggesting that the strength of biological activities of a natural product is dependent on the diversity and quantity of such constituents. Therefore, simultaneous determination of the compounds those are possibly responsible for any biological activity would facilitate decision-making process as in the selection of the plants for in-depth future investigation.

The present study is therefore undertaken to study the phytochemical and antibacterial screening of *B. variegata* (leaf, stem bark, flower) which could be used as one of the parameter for the standardization of the crude drug.

2. Material and methods

All the chemicals and solvents used in experiment were of analytical grade.

2.1. Plant collection and identification

Fresh plant parts were collected randomly from local herbal botanical garden of Bhopal, Madhya Pradesh, India. The taxonomic identities of the plant *B. variegata* were confirmed by botanist Dr. S.S. Khan (Voucher Specimen No: SP/101/LGOB/2006), Department of Botany, Safia Science College, Bhopal, Madhya Pradesh, India. Fresh plant materials were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

2.2. Preparation of extracts[10,11]

The dried plant material was pulverized into fine powder using a grinder (mixer). About 50 g of powdered material was extracted in separating funnel apparatus with 250 mL of 50% methanol solvent. The extracts obtained with each solvent were filtered through Whatman filter paper No. 1 and the water bath mantle. The sticky greenish-brown substances were obtained and stored in refrigerator for prior to use.

2.3. Protocol for measurement of preliminary phytochemical screening[10,12–17]

Standard screening tests of methanolic extracts were carried out for various plant constituents. The crude extracts were screened for the presence or absence of secondary metabolites such as alkaloids, steroidal compounds, phenolic compounds, flavanoids, saponin, tannins using standard procedures.

2.3.1. Test for carbohydrates and glycosides

2.3.1.1. Molish test

A total 2 mL of filtrate with two drops of alcoholic solution of α -naphthol were added, the mixture was shaken well and

1 mL of concentrated sulphuric acid was added slowly along the test tube and allowed to stand. A violet ring indicated the presence of carbohydrates.

2.3.1.2. Fehling test

One milliliter of filtrate was boiled on water bath with 1 mL each of Fehling solution A and B. Red precipitate in A indicates the presence of sugar.

2.3.2. Detection of glycosides

Borntrager's test was used, 200 mg crude extract was mixed with 2 mL of dilute sulphuric acid and 2 mL of 5% aqueous ferric chloride solution, boiled for 5 min which lead to oxidation to anthroquinones, indicating the presence of glycosides.

2.3.3. Test for proteins and amino acids

2.3.3.1. Biuret test

An aliquot of 2 mL of filtrate was treated with one drop of 2% copper sulphate solution. To this 1 mL of ethanol (90%) was added, followed by excess of potassium hydroxide pellets. Pink colour in ethanol layer indicated the presence of proteins.

2.3.3.2. Ninhydrin test

Two drops of ninhydrin solution were added to 1 mL of aqueous filtrate. A characteristic purple colour indicated the presence of amino acids.

2.3.4. Test for alkaloids

2.3.4.1. Mayer's test

Crude extract was mixed with Mayer's reagent (potassium mercuric iodide solution). Cream colour precipitate was formed, indicating the presence of alkaloids.

2.3.4.2. Dragondroff's test

Crude extract was mixed with Dragondroff's reagent (potassium bismuth iodide solution). Reddish brown precipitate was formed which suggested the presence of alkaloids.

2.3.5. Test for phytosterol

In chloroform test, 0.5 g of extract was treated with 10 mL chloroform and filtered. The filtrate was used to test the presence of phytosterols and triterpenoids. The extract was refluxed with solution of alcoholic potassium hydroxide till complete saponification has taken place. The mixture was diluted and extracted with ether. The ether layer was evaporated and the residue was tested for the presence of phytosterol. The residue was dissolved in few drops of dilute acetic acid, 3 mL of acetic anhydride followed by few drops of concentrated sulphuric acid. Appearance of bluish green color shows the presence of phytosterol.

2.3.6. Tests for steroidal compounds and triterpenoids

2.3.6.1. Salkowski's test

Crude extract was mixed with few drops of acetic anhydride, boiled and cooled, conc. H₂SO₄ was then added from the sides of the test tube. A brown ring at the junction of two layers was formed. The upper layer turned green which showed the presence of steroids and formation of deep red colour indicated the presence of triterpenoids.

2.3.6.2. Lieberman's test

Crude extract was mixed with chloroform and a few drops of conc. H₂SO₄, shaken well and allowed to stand for some time. Red color appeared at the lower layer indicated the presence of steroids and formation of yellow coloured layer indicated the presence of triterpenoids.

2.3.7. Tests for flavanoids

2.3.7.1. Tests for free flavanoids

A total 5 mL of ethyl acetate was added to a solution of 0.5 g of the extract in water. The mixture was shaken, allowed to settle, and inspected for the production of yellow colour in the organic layer, which is taken as positive for free flavanoids.

2.3.7.2. Lead acetate test

To a solution of 0.5 g extract in water, about 1 mL of 10% lead acetate solution was added. Production of yellow precipitate is considered as positive for flavanoids.

2.3.7.3. Reaction with sodium hydroxide

Dilute sodium hydroxide solution was added to a solution of 0.5 g of the extract in water. The mixture was inspected for the production of yellow colour which considered as positive test for flavanoids.

2.3.8. Tests for saponin

In the Froth test, 0.5 g extracts were dissolved in 10 mL of distilled water for about 30 seconds. The test tube was stoppered and shaken vigorously for about 30 seconds. The test tube was allowed to stand in a vertical position and observed over 30 min. If a "honey comb" froth above the surface of liquid persists after 30 min, the sample is suspected to contain saponin.

2.3.9. Test for tannins

2.3.9.1. Ferric chloride test

A portion of the extracts were dissolved in water. The solution was clarified by filtration; 10% ferric chloride solution was added to the clear filtrate. This was observed for a change in colour to bluish black.

2.3.9.2. Formaldehyde test

To a solution of about 0.5 g extract in 5 mL water, three drops of formaldehyde and six drops of dilute hydrochloric acid were added. The resulting mixture was heated to boiling for 1 min and cooled. The precipitate formed (if any) was washed with hot water, warm alcohol, and warm 5% potassium hydroxide successively. A bulky precipitate, which leaves a coloured residue after washing, indicated the presence of phlobatannins.

2.3.9.3. Test for phlobatanins

Deposition of a red precipitate when an aqueous extract of the plant part was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

2.3.9.4. Modified iron complex test

To a solution of 0.5 g of the plant extract in 5 mm of water a drop of 33% acetic acid and 1 g sodium potassium tartarate was added. The mixture was warmed and filtered to remove any precipitate. A 0.25% solution of ferric ammonium citrate was added to the filtrate until no further intensification of colour is obtained and then boiled. Purple or blackish precipitates, which are insoluble dilute ammonia, denotes the presence of in hot water, alcohol, or dilute ammonia, denotes the presence of pyrogallol tannin.

2.3.10. Test for phenolic compound

Ferric ferrocyanide reagent for phenolics test: 10% iron chloride (FeCl_3) (aq) was mixed with iron cyanide (FeCN_6) (1 g/100 mL or 0.1 g/10 mL), 0.1 g of ferric chloride and 0.1 g of potassium ferricyanide ($\text{K}_3\text{Fe(CN)}_6$) was freshly prepared by dissolving in 10 mL of distilled water. Equal portions of 1 and 2 was mixed, sprayed to the plates and heated at 110 °C. Change of colour to blue (instant) indicates the presence of phenolics.

2.3.11. Tests for fixed oils and fats

2.3.11.1. Stain test

The small quantity of crude extract was pressed between two filter papers; the stain on 1st filter paper indicated the presence of fixed oils.

2.3.11.2. Saponification test

In small quantity of crude extract few drop of 0.5 N of alcoholic potassium hydroxide were added to which a drop of phenolphthalein was added separately and heated in a water bath for 1 h. The formation of soap indicated the presence of fixed oils and fats.

2.4. Determination of antibacterial activity^[13,18]

2.4.1. Bacterial strains

The hydromethanolic extracts of leaves, stem bark and flower of *B. variegata* of 1000 mg/mL, 750 mg/mL, 500 mg/

mL and 250 mg/mL concentrations were tested against gram positive *Bacillus subtilis* (*B. subtilis*) (ATCC 11778), *S. aureus* (ATCC 25923), *Streptococcus epidermidis* (*S. epidermidis*) (ATCC 24676) and Gram-negative *E. coli* (ATCC 25922), *Shigella flexneri* (*S. flexneri*) (ATCC 11435), *Pseudomonas aeruginosa* (*P. aeruginosa*) (ATCC 17440) for their antimicrobial activity. All the bacterial strains were obtained from National Chemical Laboratory, Pune, India. The bacteria were grown in the nutrient broth at 37 °C and maintained on nutrient agar slants at 4 °C.

2.4.2. Preparation of inoculums (Muller Hinton media)

One single colony of each type of microorganism (from the nutrient agar stock culture) was taken with a sterile loop, and was transferred into 10 mL sterile nutrient broth. The broth cultures were incubated in a shaking incubator at 37 °C for 16–20 h.

2.4.3. Antibacterial susceptibility test: disc diffusion assay

The antimicrobial activity of crude methanolic extracts of plants were initially assessed against the three tested microorganisms using the agar diffusion method as recommended by the Clinical Laboratory Institute. Nutrient agar medium was prepared by suspending nutrient agar (Merck) 20 g/L in distilled water. The pH value of the media was adjusted to 7.0, autoclaved and allowed to cool up to 45 °C. The media was seeded with 10^6 CFU/mL prepared inoculum. Subsequently, the seeded medium (75–80 mL) was poured into pre-labelled Petri plates (diameter=14 cm) and allowed to solidify. Impregnated disks were prepared by the addition of four different concentrations *i.e.*, 1000 mg/mL, 750 mg/mL, 500 mg/mL and 250 mg/mL different plant extracts (w/v; mg/mL) to “susceptibility blank disks” (Oxoid). These were subsequently applied to the inoculated agar plates and then incubated 24 h at 37 °C. Antibacterial activity was indicated when clear inhibition zones were noted around the discs.

The diameter of the inhibition zones was measured and the results were expressed as mean of three independent experiments. The test was repeated three times. Each extract was dissolved in 99.9% dimethyl sulfoxide (Sigma-Aldrich USA) to get 100 mg/mL concentration. Standard antibiotics (5 µg) Gram-positive (TE-tetracycline, OF-ofloxacin, AZ-azithromycin, PC-piperacillin) and Gram-negative FU-nitrofurantoin, GM-gentamicin, CX-cefotaxime, NF-norfloxacin, (5 µg/disc) were prepared as positive control. Pure dimethyl sulfoxide (99.9%) was used as negative control.

3. Results

The phytochemical tests revealed the presence of flavonoids, saponins, alkaloids, fatty acid, tannins glycosides in methanolic extract of *B. variegata* (Table 1).

Table 1

Phytochemical screening of solvent extracts of *B. variegata* L. (leaf, stem bark and flower).

S. No.	Name of tests	Tests/reagents	BVL	BVB	BVF
1	Carbohydrates	Fehlings test	+	+	+
		Molish test	+	-	-
2	Glycosides	Borntrager's	+	+	+
3	Alkaloids	Dragendorff's test	-	+	+
		Mayer's test	-	-	+
4	Phytosterol	chloroform	-	-	-
5	Steroidal compounds	Salkowski's test	-	-	-
		Lieberman's test	-	-	-
6	Saponins	Froth test	-	+	+
7	Tannins	Ferric chloride test	+	+	+
		Formaldehyde test	+	+	+
		Test for phlobatanins	+	+	+
8	Fixed oils and fats	Spot test	+	-	+
		Test for free flavanoids	+	+	+
9	Flavonoids	Lead acetate test	+	+	+
		Sodium hydroxide	+	+	+
		Ferric chloride test	+	+	+
10	Phenolic compound	Ferric chloride test	+	+	+
		Biuret test	+	+	+
11	Protein and amino acid	Ninhydrin test	+	+	+

+ : presence, - : absence, BVL: *B. variegata* leaf, BVB: *B. variegata* stem bark, BVF: *B. variegata* flower

The antibacterial activity of *B. variegata* extract was assayed *in vitro* by agar disc diffusion method against Gram-positive and negative bacteria. Tables 2–6 summarizes the microbial growth inhibition of hydromethanolic extracts of

the screened different parts of *B. variegata*.

Table 5

Antibacterial of standard antibiotics to Gram-positive bacteria in different concentrations of antibiotic (5 µg/disc).

Name of the organisms	Zone of inhibition in mm			
	TE	OF	AZ	PC
<i>B. subtilius</i>	14	16	18	14
<i>S. epidermidis</i>	16	18	17	17
<i>S. aureus</i>	15	16	16	14

Table 6

Antibacterial of standard antibiotics to Gram-negative bacteria in different concentrations of antibiotic (5 µg/disc).

Name of the organisms	Zone of inhibition in mm			
	FU	GM	CX	NF
<i>E. coli</i>	12	16	8	16
<i>P. aeruginosa</i>	14	13	18	20
<i>S. flexneri</i>	18	18	12	21

3.1. Phytochemical screening

The phytochemical tests revealed the presence of flavonoids, saponins, alkaloids, fatty acid, tannins glycosides in methanol extract. The results of phytochemical screening are given in Table 1.

3.2. Antibacterial activity

The Tables 2–6 shows the zone of inhibition (in mm) values of *B. variegata* extracts of all tested microorganisms. All the

Table 2

Effect of *B. variegata* leaves extract in antibacterial activity.

Test sample concentration (mg/mL)	Gram-positive			Gram-negative		
	<i>B. subtilius</i>	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>E. coli</i>	<i>S. flexneri</i>	<i>P. aeruginosa</i>
1000	9.0	8.0	8.5	10.1	9.0	8.0
500	8.5	7.5	8.0	10.0	8.0	8.0
750	7.5	7.0	7.5	7.0	7.0	7.0
250	7.0	6.5	7.0	6.5	6.5	6.5

Table 3

Effect of *B. variegata* stem bark extract in antibacterial activity.

Test sample concentration (mg/mL)	Gram-positive			Gram-negative		
	<i>B. subtilius</i>	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>E. coli</i>	<i>S. flexneri</i>	<i>P. aeruginosa</i>
1000	17	12	12	18	13	11
500	14	10	11	14	12	10
750	11	8	10	12	10	9
250	10	6	6	11	9	8

Table 4

Effect of *B. variegata* flower extract in antibacterial activity.

Test sample concentration (mg/mL)	Gram-positive			Gram-negative		
	<i>B. subtilius</i>	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>E. coli</i>	<i>S. flexneri</i>	<i>P. aeruginosa</i>
1000	18	15	12	19	15	10
500	16	12	16	14	13	14
750	14	10	13	15	12	11
250	10	9	10	13	14	13

extracts of *B. variegata* showed considerable antibacterial activity at all the four concentrations 1000 mg/mL, 750 mg/mL, 500 mg/mL, 250 mg/mL. Tables 2–6 shows susceptibility pattern of the hydromethanolic extracts of *B. variegata* and standard antibiotic discs against some Gram-positive and Gram-negative bacteria studied. The results of antibacterial activity are reported in below:

4. Discussion

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay. Bacterial resistance to antibiotics has become a serious problem of public health that concerns almost all antibacterial agents and that manifests in all fields of their application. Novel antimicrobial compounds against new bacterial targets and drug resistance mechanisms are urgently needed. Plant derived antibacterials are always a source of novel therapeutics.

Medicinal herbs possess curative properties due to the presence of various complex chemical substance of different composition, which are found as secondary plant metabolites in one or more parts of these plants^[19]. There is continuous and urgent need for discovery of new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because of alarming increase in the incidence of new and re-emerging infectious diseases^[20].

Our earlier report on preliminary phytochemical studies of the partitioned portions showed the presence of anthraquinones derivatives, cardenolides and cardiac glycosides, flavonoids, resins, saponins and tannins. These are compounds that are known to have various sort of curative effects against most pathogenic organisms as reported by many researchers^[21,22].

These principles have been known for many years to exhibit biological activity, such as effects on the central nervous system, and antibacterial, antitumor, and anthelmintic activity have reported oils, alkaloids and anthraquinones associated with plants to have medicinal value^[23]. Others are triterpenoids, which include: cardiac glycosides, sterols, saponins and triterpenes. Mode of action of compounds present in the extracts indicates that the extracts from these plants have the potential of solving the problem of multi-drug resistance.

Maximum activity was conferred against gram positive and negative bacteria for *B. variegata* stem bark and flower extracts as compared leave extract respectively. In the present era, plant and herb resources are abundant, but these resources are dwindling fast due to the onward march of civilization. Although a significant number of studies have been used to obtain purified plant chemical, very few screening programmes have been initiated on crude plant materials. It has also been widely observed and accepted

that the medicinal value of plants lies in the bioactive phytochemicals present in the plants^[24].

The phytochemical tests revealed the presence of flavonoids, saponins, alkaloids, fatty acid, tannins glycosides in methanol extract. These phytochemical constituents are good source of antimicrobial and antioxidant activity^[25].

In conclusion, *B. variegata* extracts possess a broad spectrum of activity against a panel of bacteria responsible for the most common bacterial diseases. The preliminary results obtained in these experiments pave the road to explore the potential development of new compounds to be launched in the pharmaceutical market filling a tremendous gap, as day by day new multiresistant microorganisms emerges.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The author is thankful to Dr. R.C. Agrawal and Research staff of Jawaharlal Nehru Cancer Hospital and Research Centre, Bhopal (M.P), India for their contribution in the piece of study. This study was supported by DST, New Delhi, (Grant No. DST No. Letter No. SSD/SS/010/2010)

Comments

Background

In recent years, many possible sources of natural antibiotics have been identified and in use for several infectious diseases, mostly bacterial and fungal, but as the diseases are growing and infectious agent changing the need of newer and natural antimicrobial agents has become ardently necessary. In view of this, the searches for new antimicrobial and phytochemical agents from medicinal plants are needed. It is believed that plants which are rich in a wide variety of secondary metabolites belonging to chemical classes are generally superior in their antimicrobial activities.

Research frontiers

The purpose of this study was to carry out preclinical evaluation of popular medicinal plant species, *i.e.*, biological and phytochemical screening with particular emphasis on those compounds of which very little scientific information is available on locale specific species, in the areas intended for the investigation. This study facilitated the selection of plants with relatively high level of potency of a natural product.

Related reports

Medicinal herbs possess curative properties due to the presence of various complex chemical substance of different composition, which are found as secondary plant metabolites in one or more parts of plants. In this context these plant compounds that are known to have various sort of curative effects against most pathogenic organisms as reported by many researchers (Geidam *et al.*, 2007).

Innovations & breakthroughs

The present study is the continuation of a program aimed at investigation of antimicrobial and phytochemical properties of *B. variegata* extract to justify the traditional claim endowed upon this herbal drug as a rasayana in Ayurveda. Data regarding the antimicrobial and phytochemical properties of the plant is very significant and has been reported to have high positive results in all groups.

Applications

The results of the present study further strengthen the claim as per Ayurved and suggest the antimicrobial and phytochemical properties of plant. A drug development programme should be undertaken to develop modern drugs with the compounds isolated from plant. Proper clinical applications may also be performed, so that the natural drug may be developed and thus can be used for the welfare of the mankind.

Peer review

It is a good study in which the author evaluated the antimicrobial and phyto-chemical properties of the well known traditional plant that is found to have good antimicrobial activity. The result is very interesting and is significant in terms of qualitative authentication of the age old claim. The result and such documentation are very important for natural drug development as well.

References

- [1] Hancock RE, Nijnik A, Philpott DJ. Modulating immunity as a therapy for bacterial infections. *Nat Rev Microbiol* 2012; **10**: 243–254.
- [2] Dionisi HM, Lozada M, Olivera NL. Bioprospection of marine microorganisms: biotechnological applications and methods. *Rev Argent Microbiol* 2012; **44**: 49–60.
- [3] Benko-Iseppon AM, Crovella S. Ethnobotanical bioprospection of candidates for potential antimicrobial drugs from Brazilian plants: state of art and perspectives. *Curr Protein Pept Sci* 2010; **11**: 189–194.
- [4] Peterson LR, Dalhoff A. Towards targeted prescribing: will the cure for antimicrobial resistance be specific, directed therapy through improved diagnostic testing? *J Antimicrob Chemother* 2004; **53**: 902–905.
- [5] Bibi Y, Nisa S, Chaudhary FM, Zia M. Antibacterial activity of some selected medicinal plants of Pakistan. *BMC Complement Altern Med* 2011; **11**: 52.
- [6] Abebe D, Debella A, Urga K. *Illustrated checklist, medicinal plants and other useful plants of Ethiopia*. Addis Ababa, Ethiopia: Ethiopian Health and Nutrition Research Institute; 2003.
- [7] Cowan MM. Plant products as antimicrobial agents. *Clin Microbiol Rev* 1999; **12**: 564–582.
- [8] India Ayurvedic Pharmacopoeia Committee. *The ayurvedic pharmacopoeia of India*. New Delhi: Controller of Publications; 2003, p. 321–322.
- [9] Mali RG, Mahajan SG, Mehta AA. Rakta khachan (*Bauhinia variegata*): chemistry, traditional and medicinal uses—a review. *Pharmacogn Rev* 2007; **1**: 314–319.
- [10] Deepa P, Kaleena PK, Valtivittan K, Girish Kumar CP. Phytochemical screening and antimicrobial activity of *Sansevieria roxburghiana* Schult. and Suhult. F. *Middle-East J Sci Res* 2011; **10**(4): 512–518.
- [11] Savithamma N, Linga Rao M, Suvrulath D. Screening of medicinal plant for secondary metabolites. *Middle-East J Sci Res* 2011; **8**(3): 579–584.
- [12] Uddin G, Rauf A, Arfan M, Ali M, Qaisar M, Saadiq M, et al. Preliminary phytochemical screening and antioxidant activity of *Bergenia ciliata*. *Middle-East J Sci Res* 2012; **11**(8): 1140–1142.
- [13] Uddin G, Rauf A, Qaisar M, Latif A, Ali M. Preliminary phytochemical screening, antimicrobial activity of *Hedera helix* L. *Middle-East J Sci Res* 2011; **8**: 198–202.
- [14] Nisar M, Ali S, Qaisar M. Preliminary phytochemical screening of flowers, leaves, bark, stem and roots of *Rhododendron arboretum*. *Middle-East J Sci Res* 2011; **10**(4): 472–476.
- [15] Uddin G, Rauf A. *In vitro* antimicrobial, profile of *Pistacia integerrima* galls stewart. *Middle-East J Sci Res* 2012; **1**(2): 36–40.
- [16] Usman R, Khan A, Gul S, Rauf A, Muhammad N. Evaluation of *in vitro* antioxidant properties of selected medicinal plants. *Middle-East J Sci Res* 2012; **1**(2): 28–31.
- [17] Usman R, Khan A, Gul S, Rauf A, Muhammad N. Preliminary anti-oxidant profile of Middle-East selected medicinal plants of Pakistan. *Middle-East J Sci Res* 2012; **1**(2): 24–27.
- [18] Clinical and Laboratory Standards Institute (CLSI). *Performance standards for antimicrobial susceptibility testing*. Wayne, PA: CLSA; 2003.
- [19] Patil SB, Naikwade NS, Magdum CS. Review on phytochemistry and pharmacological aspects of *Euphorbia hirta* Linn. *J Pharm Res Health Care* 2009; **1**: 113–133.
- [20] Parekh J, Chanda SV. Antibacterial activity of aqueous and alcoholic extracts of 34 Indian medicinal plants against some *Staphylococcus* species. *Turk J Biol* 2008; **32**: 63–71.
- [21] Usman H, Musa YM, Ahmadu AA, Tijjani MA. Phytochemical and antimicrobial effects of *Chrozophora senegalensis*. *Afr J Tradit Complement Altern Med* 2007; **4**(4): 488–494.
- [22] Geidam YA, Usman H, Abubakar MB, Ibrahim B. Effects of aqueous leaf extracts of *Psidium guajava* on bacteria isolated from the navel of day-old chicks. *Res J Microbiol* 2007; **2**(12): 960–965.
- [23] Harborne JB. *Phytochemical methods; a guide to modern techniques of plant analysis*. London: Chapman and Hall; 1984.
- [24] Veermuthu D, Muniappan A, Savarimuthu I. Antimicrobial activity of some ethnomedicinal plants used by paliyar tribe from Tamil nadu, India. *BMC Complement Altern Med* 2006; **6**: 35.
- [25] Maurya R. Chemistry and pharmacology of *Withania coagulans*: an Ayurvedic remedy. *J Pharm Pharmacol* 2010; **62**: 153–160.