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Estimation of Phytochemicals, Inorganic Profile and Antimicrobial Activity of *Taxus baccata* Shoots

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The present study was designed to investigate various extracts of *Taxus baccata* shoots for photochemical screening, antimicrobial activities and heavy metal analysis. Various extracts prepared from *Taxus baccata* shoots with n-hexane, dichlromethane, Iso-butanol, water and crude extract were tested for their antimicrobial potential. These extract were tested against ten microorganism including six gram-negative bacterial strains i.e. *Escherichia coli, Pseudomonas aeruginosa, Salmoneella typhi, Kleibsiella pneumoniae, Erwinia carotovora, Agrobacterium tumifaciens,* three gram-positive bacterial strains viz *Staphylococcus aureus, Bacillus subtilis*, *Bacillus atropheous* and a fungal strain viz *Candida albicans*. Isobutanol extract showed marked antimicrobial potential against *Bacillus atropheous.* Phytochemical potential of extract was also evaluated by carrying out qualitative and quantitative analysis as well as ash value content. The qualitative tests revealed the presence of alkaloids, saponins, steroids, terpenoids and flavonoids. However tannins were not found. The quantitative tests detected alkaloids (0.2%) and saponins (4.2%). The ash value was found to be 2.68%. Heavy metal analysis was carried out to determine (Cd), (Mn), (Pb), (Cr), (Sb), (Na), (K), (Ca), (Cu) and (Fe). The metallic screening results showed the presence of Ca and K as major metallic content yielding 10021 and 8790.4 mg/kg respectively as compared to the rest of the nutrients, while Cd and Pb were not detected. The reported antimicrobial potential, phytochemicals screening and metal contents found in the samples of *Taxus baccata* shoots indicate that the plant has great pharmacological significance.

Key words: Secondary metabolites, heavy metals, antibacterial activity**.**

Plants have been widely used for variety of medicinal as well as aromatic purposes. The physicochemical and biochemical properties as well as the use of these medicinal plants are of prime importance to the health of individual and community. However most of them still remain mystery (in terms of pharmacological characterization) which needs to be explored for medicinal purposes[1]. Medicinal plants are of more importance than ever because they have the ability of producing important beneficial products for mankind especially in the field of pharmacology and medicine. The powerful medicinal agents are present in the phytochemical constituents that show specific pharmacological action on human body[2]. Some of the important bioactive phytochemicals are glycosides, flavonoids, alkaloids, saponins, phenolic compounds and tannins[3]. These important natural compounds form a variety of basic modern drugs[4]. In medicinal plants, vegetables and fruits are the natural phytochemical compounds that defend against diseases. These days, due to the haphazard use of commercial antimicrobial drugs commonly used in

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the cure of infectious diseases multiple drug resistance has been developed. In addition to this problem, antibiotics have unfavorable effects on immune suppression, hypersensitivity and allergic reactions. For the stated reasons it is mandatory and compulsory for scientists to search for new antimicrobial products. Given the shocking prevalence of antibiotic-resistant bacteria, there is a steady need for new and efficient therapeutic agents. Therefore, it is now important to produce alternative antimicrobial drugs from medicinal plants for the healing of infectious diseases[5]. Antimicrobial of plants have vast therapeutic capability. In the treatment of infectious diseases they are valuable[6, 7]. The medicinal plants also exhibit the ability to accumulate compounds present in the atmosphere, soil and water. These compounds are possibly heavy metals, explosive hazardous wastes, petroleum products and the combustible substances (8) that are discharged to the environment through automobile exhaust, industrial activities, burning and pesticides used in agriculture etc. The major constituents of these compounds are the inorganic contamination caused by heavy metals. The heavy metal is defined as those metals which have specific weights greater than 5 g/cm^3 [9]. There are total 35 metals out of which 23 are the heavy elements or "heavy metals" that are Cd, Cr, Au, Fe, Pb, Mn, Co, Cu, Ga, Hg, Ni, Pt, Sb, As, Bi, Ag, Ti, Sn, V and Zn[10]. The heavy metal analysis in the plants is very important due to the fact that they could induce toxic effect on humans and animals who consume them as such or their derived products. Small amounts of these elements are common in our diet and are in fact necessary for good health, but large amounts of any of them may cause severe or unrelieved poisoning. Heavy metal toxicity causes mental and central nervous disorders, lower energy levels and damage to kidneys, liver, blood composition, lungs and other organs. Slowly progressing physical, muscular, and neurological degenerative processes that mimic Parkinson's disease, muscular dystrophy, Alzheimer's disease and multiple sclerosis are because of long-term exposure to these metals. Repeated long-term contact with some of these metals or their compounds may even cause cancer and allergies[11].

Taxus baccata (English yew) belongs to family "taxaceae" is one of the important medicinal plant. Its leaves have been used in traditional medicine for antimalarial, antirheumatic and bronchitis[12]. In Ayurvedic medicine it was known as Talispatra that was used as an antispasmodic, seadative and aphrodisiac as well as against asthama. It was also listed in Avicenna's cardiac drugs namely Zarnab[13]. All the parts of this plant except the arillus part enveloping the seeds contain toxic taxine alkaloids which cause poisoning in human and animals[14]. *Taxus baccata* shoots has shown promising data for being a suitable bio indicator of polluting metals and organic compound. Atmospheric metal concentration monitoring can be carried out on the *Taxus baccata* tree sample to illustrate heavy metal distribution trends as tree bark and leaves are known to absorb and accumulate air born contaminates[15]. Keeping in view the importance of *Taxus baccata*, the present study was aimed to evaluate the phytochemicals and antimicrobial activity of the different extracts of *Taxus baccata* shoots. This investigation is much more valuable as the actual nutrient contents of the medicinal plants in terms of the essential trace elements are also identified to find the inorganic profile.

MATERIALS AND METHODS

Collection of plant

The *Taxus baccata* shoots were collected from Mahandri washed with running water and finally by de-ionized water and transferred to proper container. The *Taxus baccata* shoots were dried in shade and converted into powder for further analysis.

Extraction of plant material

The pulverized plant material was extracted at room temperature with 80% methanol for thorough extraction. The process was repeated three times allowing it to soak for at least 3 days using maceration method. Later on the crude extract was concentrated using vacuum rotary evaporator (BUCHL rolavapour R-200). After concentration the extract was stored in sample bottle.

Fractionation /solvent-solvent extraction

The crude extract was fractionized on the basis of miscibility and different polarity using various organic solvents like n-hexane, dichloromethane, Iso-butanol and water fractions. After that each fraction was concentrated and dried

on vacuum rotary evaporator (BUCHL rolavapour R-200) under reduced pressure.

Phytochemical screening

Chemical test to identify the bioactive chemical constituents were carried out on the methanolic extracts following standard procedures described by (16).

Alkaloid test

0.2g of extract was warmed with 2% sulphuric acid for 2 minutes. After filtration a few drops of Dragendroff's reagent was added to the filtrate. Appearance of orange red precipitate indicated the presence of alkaloid.

Saponin test

About 0.2g of the extract was shaken with distilled water (5ml) and heated to boil. The formation of frothing indicated the presence of saponins.

Flavonoids Test

1.5 mL of 50% aqueous methanol was added to 4 ml of plant extracts. After warming the solution magnesium metal was added. Then 5-6 drops of concentrated HCl was added to that solution. Appearance of red color indicated the presence of flavonoids (17).

Steroid test

The extract was dissolved in 2ml of acetic acid anhydride and added with 2ml of concentrated sulphuric acid. The color change from violet to green indicated the presence of steroids.

Terpenoid Test

Extract (0.2g) was added with 2ml chloroform. After that 3ml of sulphuric acid was carefully added to form a layer. The formation of reddish brown color at the interface indicated the presence of terpenoids.

Tannin Test

A small quantity of the extract was mixed with water. The solution was heated on water bath and then filtered. A few drops of ferric chloride were added to the filtrate. Appearance of dark green solution indicated the presence of tannins.

Quantitative Analysis

Alkaloid Test

Dried plant material was ground into powder form. 15.67g of powder was extracted with 100% methanol (150 ml). After that the methanol was allowed to evaporate. 5g dried extract was then dissolved in 5% HCl (50ml). After that, mixture was centrifuged and the aqueous portion was transferred to a test tube and then basified with NH4OH (PH 8-10). Now the aqueous basic portion was extracted three times with CHCL3. It was then concentrated under reduce pressure. After drying the sample was weighed to determine the amount of alkaloid residue (18).

Sapoins Test

16g of ground plant material was defatted with 30ml n-hexane. After that the material was extracted three times with 30ml methanol and was concentrated to one third of its original volume. Now 100ml cold acetone was added to the extract then left into the refrigerator for 50 minutes. The extract was then filtered by pressure filtration using pre weighed whattman No 1 filter paper and the weight of the saponnis was determined.

Ash value

2.5g of plant material was burned completely to ash in a pre weighed crucible. After covering the crucible with lid it was place in a furnace at 600 p C for 2 hours. The crucible was then left to cool. After cooling, the crucible was placed in desiccators and re weighed. The %age of ash value was then calculated.

Test Microorganism

Gram negative pathogenic strains of bacteria viz *Escherichia coli* (ATCC#25922), *Pseudomonas aeruginosa* (ATCC#9721) and clinical isolates *Salmoneella typhi, Kleibsiella pneumoniae, Erwinia carotovora* and *Agrobacterium tumifaciens* along with gram positive bacterial strains of *Staphylococcus aureus* (ATCC#6538) and clinical isolates *Bacillus subtilis* and *Bacillus atropheous* as well as a fungal strain *Candida albicans* (clinical isolate) were acquired from PCSIR laboratory Peshawar, Pakistan.

Determination of Antimicrobial Activities

Antimicrobial activity of different solvent extract dichloromethane, iso-butanol, n-hexane, water and crude extract were determine by disc diffusion method on nutrient agar medium in term of diameter of inhibition zone[19] against three gram-positive, six gram-negative bacteria and one fungus . Nutrient broth (1.3g 100ml) and nutrient agar (2.8g 100ml) were prepared by dissolving in distilled water. The nutrient broth was dispensed in test tube (7-8ml) and flask (20-25ml). All the apparatus and media were sterilized at 120p C and 15psi pressure for 20 minutes. The agar media was poured

into Petri dishes and left to incubate overnight at 37p C to check any contamination. The stock culture was freshened up by streaking on sterilized agar Petri dishes and placing them in incubator overnight. After that the cultures were inoculated into flask containing broth media. The flasks were then kept in shaking water bath (Model GLSC-SBR 04-28) at 37 p C for 16 hours at 200rpm. Next day microbial cultures were standardized in test tube by comparing them with 0.5 McFarland (turbidity) standards. After that standardized microbial culture (100µl) were spread on each nutrient agar plates. These impregnated plates were left for 15 minutes in refrigerator for absorption. Whattman filter paper I disc were placed on these agar media plates. The extracts stock solutions in different concentration 1 and 2 mg/disc in 6µl and 12µl volume were applied on these discs in duplicate. Antibiotic including Clorimazole, Ciprofloxacin and Azithromycin were applied as positive control on separate plates against *Candida albican,* gram-negative bacteria and gram-positive bacteria respectively. While DMSO was used for making stock solution was applied as negative control. All the plates were then left to incubate overnight at 37p C. Measurement of zone of inhibition around each disc in mm was recorded for each extract to analyze the antimicrobial potential of different extracts.

Heavy Metal Analysis

Sample preparation

The polyethylene container and glass that were to be used for analysis were washed with tap water and then left to soak overnight in 6M HNO3 solution. 8.5g of plant sample was transferred into a silica crucible and kept in a muffle furnace for ashing at 450p C for 3 hours. 5ml of 6M HCL was added to the crucible and was kept on hot plate and digested to obtain a clear solution. The final residue was dissolved in 0.1M HNO3 solution and made volume upto 50ml (20).

Analysis

The heavy metals including Cd, Cr, Mn, Pb, Sb, Cu and Fe in plant sample were analyzed using atomic absorption spectrophotometer (HITACHI Z-8000 polarized zeman japan) while Na, K and Ca were determined by flame photometer (Jenway PFP 7, UK).

RESULTS

All the plant samples were investigated for their phytochemical constituents including solvent extractive values, ash values, antimicrobial potential and detection of heavy metals.

The table 1 data shows that the extractive value of n-hexane was found 1% followed by 1.43% in DCM , 3.28% in Iso-butanol while the highest value of 6.84% was recorded in the solvent using water.

The qualitative phytochemical analysis of methanolic extract of *Taxus baccata* shoots has been summarized in table 2. The results show the presence of alkaloids, saponins, flavonoids,

Table 1. Extractive value of *Taxus baccata* shoots in different solvents

Plant specie	Plant material (g)	Hexane(%age)	DCM(%age)	Isobutanol(%age)	Aqueous(%age)
Taxus Baccata	300	1	1.43	3.28	6.84

Table 2. Qualitative analysis of phytochemicals in *Taxus baccata* shoots

Plants specie	Alkaloid	Saponins	Flavonides	Steroids	Terpenoids	Tannins
Taxus Baccata	+ve	+ve	+ve	+ve	+ve	-ve

Table 3. Quantitative analysis of crude Phytochemical and ash value in the shoot of *Taxus baccata*

Plants species	Alkaloid % age	Saponins % age	Ash value%
Taxus Baccata	0.2	4.2	2.68

steroids and terpenoids while the tannins were found absent.

Table 3 data shows the quantitative estimation of crude phytochemicals in shoots of *Taxus baccata*. The results showed highest concentration of saponins (4.2%) as compared to that of alkaloids (0.2%), while total ash content of 2.68% was recorded.

The antimicrobial properties of the *Taxus baccata* shoots against six gram-negative bacterial strains are shown in table 4. The plant extract was effective against most of the bacterial species and fungus. However a few showed the highest activity. The Iso-butanol extract showed the best activity against *B.atropheous* (28mm in 6µl and 30mm in 12µl). On the other hand hexane extract was found inactive against *E.coli, S.typhi, S.aureus*, and *P.aureginosa*. The *S.typhi, S.aureus, E.carotovora, C.albican, A.tumificians* and *P.auregonosa* showed resistance against water extract. The crude extract showed the highest activity (29mm in 6µl) against *B.atropius* while found less active (10mm in 6µl) against *E.coli*. No zone of inhibition was recorded against *S.aureus* like that of crude extract and aqueous extract. In case of fungal activity, the DCM extract showed the highest zone of inhibition (18mm in 12µl) against *C.albican* while the least value for crude extract (12mm in 6µl) was found against the same organism. The aqueous extract was found totally inactive and showed no zone of inhibition.

The results of heavy metal analysis of the *Taxus baccata* shoots are listed in table 6. A total of 10 metals including (Cd, Mn, Pb, Cr, Sb, Cu, Fe, Ca, Na, K) were determined by atomic absorption spectrophotometer and flame photometer.

As can be seen from table No 6, the highest concentration of Ca (10021 mg/kg) was present in the shoot of *Taxus baccata* followed by K (8790.4 mg/kg). The lowest concentration 0.58 mg/kg of Cr was found in the *Taxus baccata* shoots while the Cd and Pb were not detected in the same sample.

Table 4. Antimicrobial activity of Shoots of *Taxus baccata*

Organism	Zone of inhibition in mm										Antibiotics	
	Hexane		DCM		Isobutanol		Aqueous		Crude extract		Standard Drugs	Inhibition (mm)
	6µl	12µl	6µl	12µl	6µl	12µl	6µl	12µl	6µl	12µl		
E.coli	Nil	Nil	19	20	13	15	14	15	10	11	Ciprofloxacin	37
S.Typhi	Nil	Nil	17	18	13	14	Nil	Nil	14	12	Ciprofloxacin	23
E.carotovora	12	16	20	22	14	19	Nil	Nil	12	14	Ciprofloxacin	17
A.tumifaciens	14	15	20	22	15	17	Nil	Nil	14	20	Ciprofloxacin	25
P.aeuroginosa	Nil	Nil	24	22	15	18	Nil	Nil	17	18	Ciprofloxacin	34
K. Pneumoniae	15	18	24	25	15	19	15	20	20	19	Ciprofloxacin	29
S. aureus	Nil	Nil	17	15	17	19	Nil	Nil	Nil	Nil	Azithromycin	21
B.subtilis	13	14	15	18	20	21	13	20	19	18	Azithromycin	23
B.atropheous	20	24	25	27	28	30	20	21	29	26	Azithromycin	27
C.albican	13	14	15	18	13	15	Nil	Nil	12	13	Clotrimazole	32

Table 5. Instrumental conditions of the atomic absorption spectrophotometer

Metals	Cd	Cr	Mn	Pb	Sb	Cu	Fe
Wavelength (nm)	228.8	359.3	279.5	283.3	217.6	324.8	248.3
Slit width (nm)	1.3	1.3	0.4	1.3	0.4	1.3	0.2
Burner height (mm)	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Light source	Hollow cathode lamp						
Flame	Air Acetylene						

DISCUSSION

Phytochemicals are secondary metabolites that are used for different types of ailments. The phytochemical screening and quantitative analysis of extracts of *Taxus baccata* shoots revealed that the shoots were rich in saponins and some alkaloids. Both of these are known to exhibit physiological as well as medicinal activities. The results showed high content (4.2%) of saponins, which being the important plant constituent are the main group of glycosides (21). Saponins are also believed to be useful in the human diet for controlling cholesterol, but some are poisonous if swallowed and can cause urticaria (skin rash) in many people. The presence of saponins in the *Taxus baccata* shoots could be responsible for its hypotensive, antihyper cholesterol and cardiac depressant properties (22). Saponins exhibit anticancer and anthelmintic activities. It also shows antidiarrhoeal activity by inhibiting histamine release in vitro (23). Besides saponins, the phytochemical analysis also detected the presence of alkaloids but in lower concentration of 0.2%. Like saponins alkaloid is one of the largest groups of chemical arsenals produced by plants. These are heterocyclic nitrogeneous compounds. Alkaloids have detoxifying and anti hypertensive properties (24). Alkaloids show anthelmintic, antidiarrhoeal and antimicrobial activities. Antidiarrhoeal activity possesses anti-oxidating effects by reducing nitrate generation which is useful for protein synthesis and suppresses transfer of sucrose from stomach to small intestine. Antimicrobial activity of alkaloids inhibits microbial growth by intercalating into cell wall and DNA of parasites (23). Thus the antimicrobial activity of the plant can be attributed to the presence of alkaloids. In this study the antimicrobial activity of the shoots of *Taxus baccata* was found using different solvent extracts as the methanolic extract of the plant contains active compounds of varying solubility and polarity. Thus using different solvents, compounds were separated on the basis of their solubility and polarity. The results showed that the extract was more active against gram positive bacteria as compared to gram negative bacteria, while against fugus all the extracts were found less effective. Iso-butanol extract showed higher inhibition zone against *B. atropheous* compared to the rest of the extracts. The difference can be attributed to the solubility of active components in Iso-butanol which are best active against *B. atropheous*. The aqueous extract showed negligible antibacterial activity against gram negative bacteria, gram positive bacteria and a fungus due to the fact that in aqueous portion the active components were less soluble. Such chemical compounds with antimicrobial activities have enormous therapeutic potential exhibiting effectiveness in the treatment of infectious diseases.

As the medicinal plants are very effective source of curative substances to heal various diseases, the amount of heavy metals in the *Taxus baccata* shoots was also analyzed to show the potential threat of their effects to the human beings and animals. Useful elements in plants such as manganese, potassium, calcium, sodium, magnesium and iron help the good health while toxic elements like cadmium, lead, mercury, arsenic and nickel might also be present in some plants threatening the consumer. The results of our investigation recorded the highest content of Ca and K while Cr, Cu and Sb values were significantly lower however Pb and Cd were not detected. Calcium is the main constituent of teeth and bones. It is also one of the main components of the cell membrane that controls its permeability and electrical properties, as well as plays very important role in the muscle contraction, neuro vascular transmission and blood coagulation. Normal human blood contains from 0.9~11.5 mg of calcium

Table 6. Heavy metal analysis in the shoot of *Taxus baccata*

Sr No	Metals	Resultsmg/kg
1	Cd	ND*
2	Mn	185.2
3	Pb	ND
4	Cr	0.58
5	Sb	10.67
6	Na	222.7
7	K	8790.4
8	Ca	10021.
9	Cu	3.985
10	Fe	117.2
2.	*Not detected	

per 100 ml. Similarly potassium has an important function in regulating acid base balance in the cell and water retention. It is also very essential for protein synthesis by ribosomes (25). The absence of Pb and Cd in our analysis can be attributed, along with other certain factors, to the fact that this plant grew in non polluted region. Hence this investigation of heavy metal contents shows that *Taxus baccata* shoots contain non toxic heavy metals and can be used in the form of herbal concoction and extracts for the treatment of various diseases. This study presents the pharmacological importance of the *Taxus baccata* shoots in terms of its physicochemical properties, antimicrobial potential and heavy metal contents. The data obtained will be helpful in understanding the therapeutic values and curative effects of the *Taxus baccata* by identifying its various medicinal potentials.

CONCLUSION

The aforesaid study throws light on the importance of the studied plant and this shows the use of this plant by the local quacks for different ailments. The study in process is a venture in informing the local and common populaces who use this crude phytochemicals or the plants as a whole for the cure of various diseases. This study also provides scientific basis for the above-discussed compounds. It helps as initiative for evaluating scientifically the crude phytochemical constituents and their antimicrobial activities along with micro and macronutrients. It is hoped that this study will enhance the knowledge about these substances and shows new leads for further areas of research to investigate advance pharmaceutical values of the subject plant.

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