

Screening of phytochemical compounds, trace metals and antimicrobial activity of *Anacyclus pyrethrum*

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Abstract

In the present study is phytochemicals, trace metals and antimicrobial activity of ethanolic extract of *Anacyclus pyrethrum*. The phytochemical screening of the crude ethanolic root, stem and leaf extracts showed the positive results of steroids, triterpenes, reducing sugar, sugar, alkaloids, flavonoids, saponin, tannins, anthraquinones and amino acids. The average mean concentrations of Cd, Cr, Cu, Fe, Ni, Pb and Zn in plant sample were BDL, 0.03, 0.42, 0.79, BDL, BDL and 0.58 mg kg⁻¹, respectively. The descending order of the metal content in the plant sample were: Zn > Fe > Cu > Cd > Cr = Ni = Pb. Among various part of plant extracts studied for antimicrobial activity, root ethanolic extract showed highest of inhibition than leaves and stem ethanolic extracts.

Keywords: *Anacyclus pyrethrum*, Antimicrobial activity, Phytochemistry, Trace metals.

1. Introduction

Plants are rich in nutrient compounds and they are main source of food. Plants were used for treatment of disease without knowledge about the compounds present and their mode of action. Nature has given us a very rich botanical wealth and large number of diverse types of plants grows in different parts of the country. Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs [1]. According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs.

Most of the major and minor ions present in the water and soil and were transferred from soil/water to living things [2][3]. Medical plants contain large varieties of chemical substances which possess important therapeutic properties that can be utilized in the treatment of human diseases, which have pain relieving, healing abilities and etc. Modern technique and pharmacological screening procedure results new plant drugs usually find their way into modern medicines. Now a day's antibiotic resistance in medically important microbes is the major problem

faced by the world and maximum number of plant are being screened for their possible pharmacological value. [4] The indiscriminate use of commercial antimicrobial drugs has resulted in multiple drug resistance. Antibiotics may also cause adverse effects on the host including allergies, hypersensitivity and immune-suppression.

Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases. Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously minimizing many of the side effects that are often associated with synthetic antimicrobials [5]. In this present investigation, one of the best Indian medicinal plant such as *Anacyclus pyrethrum* was selected and screened for their phytochemical, trace metal concentrations and antimicrobial activity of their root, stem and leaf extracts.

2. Materials and Methods

2.1 Sample collection

The *Anacyclus pyrethrum* plant was collected from Thanjavur district, Tamil Nadu, India during August to November 2015.

2.2 Solvent Extraction

The shade dried root, stem and leave parts of *A. pyrethrum* were collected, and coarsely powdered using a pulverizer. These powders were successively extracted with ethanol (80 °C) using soxhelt apparatus. The solvents were removed by vacuum distillation in a rotatory evaporator at 60 °C. The extract was filtered through Whatman No. 1 filter paper and concentrated on a water bath to a syrupy mass. The dried substance was dissolved in suitable solvents and stored in cold room for future use.

2.3 Phytochemical screening

2.3.1 Qualitative analysis

The solvent extracts were subjected to routine qualitative chemical analysis to identify the nature of phytochemical constituents present in sample [5].

2.3.1.1 Steroids

3 ml of test solution and minimum quantity of chloroform was added with 3 - 4 drops of acetic anhydride and one drop of concentrated H₂SO₄. Purple color thus formed changes into blue or green color indicating the presence of steroids.

2.3.1.2 Triterpenoids

3 ml of test solution was added with a piece of tin and 2 drops of thionyl chloride. Formation of violet or purple colour indicates the presence of triterpenoids.

2.3.1.3 Reducing Sugars

3 ml of test solution was added with a 2 ml of Fehling's reagent and 2 ml of water. Formation of reddish orange color indicates the presence of reducing sugar.

2.3.1.4 Sugars

3 ml of the test solution was added with very small quantity of anthrone reagent and a few drops of concentrated H₂SO₄ and heated. Formation of green or purple color indicates the presence of sugars.

2.3.1.5 Alkaloids

3 ml of test solution was taken with 2N HCl. Aqueous layer formed was decanted and then added with one or a few drops of Mayer's reagent. Formation of white precipitate or turbidity indicates the presence of alkaloids.

2.3.1.6 Phenols

3 ml of test solution in alcohol was added with one drop of neutral ferric chloride (5%) solution. Formation of intense blue color indicates the presence of phenols.

2.3.1.7 Flavonoids

3 ml of test solution in alcohol was added with a bit of magnesium and one (or) two drops of concentrated HCl and heated. Formation of red or orange color indicates the presence of flavonoids.

2.3.1.8 Saponins

3 ml of test solution was added with water and shaken. Formation of foamy lather indicates the presence of Saponins.

2.3.1.9 Tannins

3 ml of test solution was added with water and lead acetate. Formation of white precipitate indicates the presence of tannins.

2.3.1.10 Anthroquinones

3 ml of test solution was added with magnesium acetate. Formation of pink color indicates the presence of anthroquinones.

2.3.1.11 Amino Acids

3 ml of test solution was added with 1% ninhydrin in alcohol. Formation of blue or violet color indicates the presence of amino acids.

2.3.1.12 Catechins

3 ml of test solution in alcohol was added with Ehrlich reagent and a few drops of concentrated HCl. Formation of pink color indicate the presence of catechins.

2.3.2 Quantitative analysis of phytoconstituents

The chlorophyll pigments in the leaves were estimated following the method of Arnon [6]. Amino acids were estimated by ninhydrin method [7]; which is calorimetrically measured at 570nm. Proteins were estimated by Bradford method [8] and the absorbance was measured at 595nm against blank/ sample. Carbohydrates were estimated by anthrone method [9] which can be measured by using colorimetrically at 620 nm (or) by using a red filter. All the trials were performed thrice and the mean values were presented.

2.3.3 Trace metal analysis

The cleaned *A. pyrethrum* whole plant was dried in shadow area and was grinded with agate mortar and pestle. The powdered plant sample was stored in sterile plastic container. The 1 g of powdered plant sample was treated with aqua-regia mixture in Teflon bomb and was incubated at 140 °C for 2-3 days. After incubation, the reaction mixture was filtered with whatman No.1 filter paper. Then, the extraction was tested for trace metals (Fe, Cu, Zn, Pd, Cd, Cr and Ni) analysis by the 797 VA Computrace voltametry, Metrohm. To avoid the contamination, the devices were rinsed with acidified water (10% HNO₃) and weighted to dissolve metals before analysis. All the equipment and containers were soaked in 10% HNO₃ for 24 h then rinsed thoroughly in de-ionized water before use [10].

2.4 Testing of antimicrobial activity

The test strains were *Aeromonas liquefaciens* MTCC 2645 (B1), *Enterococcus faecalis* MTCC 439 (B2), *Klebsiella pneumoniae* (B3), *Micrococcus luteus* NCIM 2871 (B4), *Salmonella typhimurium* NCIM 2501 (B5), *Vibrio cholerae* (B6), *Candida albicans*

MTCC 1637 (F1), *Cryptococcus* sp. MTCC 7076 (F2), *Microsporum canis* (F3) and *Trichophyton rubrum* (F4). The cultures were obtained from MTCC, Chandigarh and NCIM, Pune, India. Microbial strains were tested for antimicrobial sensitivity using the disk diffusion method [2][4][11][12]. The antibacterial and antifungal activities of test samples were analyzed against certain microorganisms on muller hinton agar (MHA) and potato dextrose agar (PDA), respectively [2][13]. The solvent extracted samples were dissolved in concentrated DMSO. A sterile cotton swab was used to inoculate the bacterial suspension on surface of agar plate. The two different concentrations (25 and 50 µg/ml) of sample were poured into disk and placed on agar plates, separately. For negative control study, the DMSO was used. The plates were incubated at 37±1°C for 24–48 h (for bacteria) and 25 ±1°C for 48–72 h (for fungi) [3][14]. After incubation, the zone of inhibition was measured with ruler. The assays were performed in triplicate and the average values are presented. Methicillin – 10mcg (for bacteria) and Itraconazole – 10mcg (for fungus) was used as positive control [15][16]. All the media, standard discs and sterile disc were purchased from Hi-Media (Mumbai, India).

3. Results and Discussion

3.1 Phytochemical constituents of secondary metabolites

Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Phytomedicine can be used for the treatment of diseases as is done in case of Unani and Ayurvedic system of medicines or it can be the base for the development of a medicine, a natural blueprint for the development of a drug. The present study carried out phytochemical analysis on the *A.pyrethrum* medicinal plant ethanolic extract revealed the presence of steroids, triterpenes, reducing sugar, sugar, alkaloids, flavonoids, saponin, tannins, anthraquinones and amino acids in the extracts and the results were summarized in Table 1. The quantitative phytochemical results were summarized in Table 2. Secondary metabolism facilitates the primary metabolism in plants. This primary metabolism consists of chemical reactions that allow the plant to live. In order for the plants to stay healthy, secondary metabolism plays a pinnacle role in keeping all the of plants' systems working properly.

Steroids along with phospholipids function as components of cell membranes. Steroids such as cholesterol decrease membrane fluidity. Terpenes are released by trees more actively in warmer weather, acting as a natural form of cloud seeding. The clouds

reflect sunlight, allowing the forest to regulate its temperature. Alkaloids are antibacterial berberine, the anticancer compound vincristine, the anti-hypertensionagen treserpine, the cholinomimetic galantamine, the spasmolysis agent atropine, the vasodilator vincamine, the anti-arrhythmia compound quinidine, the anti-asthma therapeutic ephedrine, and the antimalarial drug quinine. Although alkaloids act on a diversity of metabolic systems in humans and other animals, they almost uniformly invoke a bitter taste [5]. Flavonoids are one class of secondary plant metabolites that are also known as Vitamin P or citrin [17]. These metabolites are mostly used in plants to produce yellow and other pigments which play a big role in colouring the plants.

Some of the trace metals are essential for plant growth whereas many of them affect the plant physiology. Especially, the role of trace metal pollutants causing injury to plants either by direct toxic effect or modifying the host physiology rendering it more susceptible to infection [18] Which leads to affects the photosynthesis process, growth and their efficiency [19]. The mean concentrations of metals such as Cd, Cr, Cu, Fe, Ni, Pb and Zn in plant sample were BDL, 0.03, 0.42, 0.79, BDL, BDL and 0.58 mg kg⁻¹, respectively (Table 3).

The results of antimicrobial activity of various solvent extracts of *A.pyrethrum* root, stem and leaves by disc diffusion method are in depicted Table 4. The two tested concentrations such as 25 and 50µg/ml produce zone of inhibition on MHA and PDA plates for bacteria and fungi, respectively. In the present study, higher (50 µg/ml) concentration of sample got greater sensitivity than (25 µg/ml) lower concentration in most of the microorganisms. In bacteria, the ethanol extract of all the samples were most effective against *Enterococcus fecalis* (B2) while the smaller effect was noticed from *Micrococcus luteus* (B4) comparing with standard/ commercial antibiotic for bacterial strains. Like in fungi, the test sample was effective against *Trichophyton rubrum* (F4). There is no antimicrobial activity in solution devoid of sample used as a vehicle control (concentrated DMSO), reflecting that antimicrobial activity was directly related to the sample.

Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Phytomedicine can be used for the treatment of diseases as is done in case of Unani and Ayurvedic system of medicines or it can be the base for the development of a medicine, and now a days many drugs were procured from different sources like marine living and non-living things including marine

plants [20]. Phytochemical constituents such as alkaloids, glycosides, reducing sugar, flavonoids, tannins, saponins, and several other organic compounds are secondary metabolites of medicinal plants that serve as defence mechanism against many microorganisms and insects [21].

Various workers have already shown that Gram positive bacteria are more susceptible towards plants extracts as compared to Gram negative bacteria. The alkaloids have been investigated for many pharmacological properties including antiprotozoal, cytotoxic, antidiabetic [22] and anti-inflammatory properties [5]. In the present study medicinal plant showing presence of alkaloids. The saponin is used as

mild detergents and in intracellular histochemistry staining to allow antibody access to intracellular proteins. In medicine, it is used in hypercholesterolemia, hyperglycaemia, antioxidant, anti- cancer, anti-inflammatory, central nervous system activities [23] and weight loss. It is also known to have antifungal properties [24]. Alternatively, the passage of the active compound through the Gram negative cell wall may be inhibited. It is thought that observed differences may result from the doses used in this study. In addition, microorganisms show variable sensitivity to chemical substances related to different resistance levels between strains [25].

Table 1: Qualitative phytochemical constituent of *A.pyrethrum*

Phytochemical Constituents	Ethanollic extraction		
	Root extraction	Stem extraction	Leaves extraction
Steroids	+	+	+
Triterpenes	+	-	+
Reducing sugars	+	-	-
Sugars	+	+	+
Alkaloids	+	+	+
Phenolics	-	-	-
Catechins	-	-	-
Flavonoids	+	+	+
Saponins	+	+	+
Tannins	+	+	+
<u>Anthraquinones</u>	+	+	+
Amino acids	+	-	+

+ = Present; - = Absent

Table 2: Quantitative phytochemical constituent of *A. pyrethrum*

Biochemical constituents	<i>A.pyrethrum</i> (mg/g)
Chlorophyll A	0.240
Chlorophyll B	0.820
Total Chlorophyll	1.060
Amino acid	160.0
Protein	3.140
Carbohydrate	1.726
Phenol	0.788

Table 3: Concentration of trace metals in *A. pyrethrum*

Sampling Site	Sample Name	Trace Metals (mg kg ⁻¹)						
		Cd	Cr	Cu	Fe	Ni	Pb	Zn
Thanjavur Tamil Nadu	<i>A.pyrethrum</i>	BDL	0.03	0.42	0.79	BDL	BDL	0.58

BDL – Below detectable limit

Table 4: Antimicrobial activity of the ethanolic solvent extracts of root, stem, and leaves of *A.pyrethrum*

S.No	Test Microorganisms	Conc. /Code	Ethanol extract µg/ml (100µl/disc)						PC	Diseases	Route of Transmission
			Root		Stem		Leaves				
	Bacteria		25	50	25	50	25	50	10 mcg		
1.	<i>Aeromonas liquefaciens</i>	B1	9	10	8	11	10	12	14	Wound Infections / Gastroenteritis	Water / Food
2.	<i>Enterococcus faecalis</i>	B2	11	12	10	12	12	13	8	Endocarditis / Epididymal Infections	Water / Food
3.	<i>Klebsiella pneumoniae</i>	B3	12	14	10	12	11	14	28	Acute diarrhoea / Dysentery	Water / Food
4.	<i>Micrococcus luteus</i>	B4	10	15	12	14	11	15	38	Skin & Pulmonary infections	Soil / Water / Air / Food
5.	<i>Salmonella typhimurium</i>	B5	12	16	12	15	13	16	0	Typhoid	Water / Food
6.	<i>Vibrio cholerae</i>	B6	12	14	11	12	10	11	16	Cholera	Water / Food
Fungi											
7.	<i>Candida albicans</i>	F1	11	14	12	14	12	15	10	Skin infection / Gastrointestinal tract Infection	Air / Wound / Soil / Water
8.	<i>Cryptococcus sp.</i>	F2	13	16	10	12	11	14	9	Bronchiectasis / Endophthalmitis.	Air / Wound / Soil / Water
9.	<i>Microsporium canis</i>	F3	13	14	12	14	13	14	9	Tinea capitis / Ringworm	Air / Wound / Soil / Water
10.	<i>Trichophyton rubrum</i>	F4	12	16	10	11	12	14	7	Tinea corporis / Tinea pedis	Air / Wound / Soil / Water

PC- Positive Control (Using antibiotic disc; Bacteria – Methicillin (10mcg/disc); Fungi – Itraconazole (10mcg/disc))

4. Conclusion

The present research work concludes that *A.pyrethrum* is important medicinal plant with varied pharmacological spectrum. The plant shows the presence of many phytochemical constituents which are responsible for antimicrobial property. The presence of heavy metals indicated that the plant was contaminated as well as which is resistant to the trace metal. Remarkably, the secondary metabolites of *A.pyrethrum* were not affected. In this endeavour, traditional herbal medicines must perforce be granted the benefits of modern science and technology to serves further global needs. The *A.pyrethrum* have potential for development of antimicrobial agents against for some human pathogens.

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