PHARMACOGNOSTIC STANDARDIZATION OF *ANACYCLUS PYRETHRUM* LINN AND ITS BIOLOGICAL STUDIES

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ABSTRACT

Despite the long history of traditionally used and clinically potential plant *Anacyclus pyrethrum* (Akarkara; family – Asteraceae) root parts, the plant has never been investigated systematically for the scientific phytochemical and biological studies till date. Thus, present investigation was envisaged to perform experiments to study phytochemical and biological activities. The various extracts and fractions were subjected to phytochemical screening. The complete profile of pharmacognostic standards of plant was established as per standardized procedures. The results revealed that the plant contain alkaloids, anthraquinone glycosides, carbohydrates, tannins and flavonoids. The thin layer chromatography of petroleum ether-, chloroform and methanol extracts showed presence of three-, seven- and five spots. Quantitatively maximum phenolic content was found in ethyl acetate fraction (6.48 % w/w) followed by methanol extract (2.40 % w/w). Various extracts and fractions of plant were screened for antioxidant activity by DPPH method, and the results revealed that maximum activity was shown by ethyl acetate fractions as compared to other test samples. In antimicrobial studies, the zone of inhibition in mm was determined by cup plate method. Amongst various test samples, only the ethyl acetate fraction showed maximum antibacterial activity against *Staphylococcus aureus* MTCC 7443, *Staphylococcus epidermides* MTCC 1133, *Pseudomonas aeruginosa* MTCC 2449 and *Escherichia coli* MTCC 1235 followed by methanol extract whereas other test samples were devoid of antibacterial activity. In antifungal study, ethyl acetate fraction exhibited maximum antibacterial and antifungal activity against *Candida albicans* MTCC 1637 and *Aspergillus niger* MTCC 1235, followed by methanol extract, whereas other test samples were devoid of antifungal activity. The research findings of the present investigations suggest that phenolic compounds might be responsible for the biological profile of plant. Further, it can be concluded that a complete monograph of plant has been established. These findings are very important for natural product scientists before performing any research work on plant.

Keywords: *Anacyclus pyrethrum*, antioxidant, Ash value, Extractive value, Phenol

INTRODUCTION

A close scrutiny of the available literature reveals that *Anacyclus pyrethrum* is widely known for its medicinal properties. The plant has a long history of use, in various traditional as well as alternative and complementary systems of therapeutics in treatment of pain, epilepsy, inflammation, edema, headache, stammering, toothache, heart disease, fever, cough, loss of digestive fire, paralysis and sciatica. The plant has been scientifically reported for various pharmacological activities such as antiepileptic, anti-inflammatory, cyclooxygenase and 5-lipoxygenase inhibition, antidiabetic, anticonvulsant, antidepressant and antiamnesic activities. The plant has been reported to contain various phytoconstituents related to different classes such as insulin, 2-phenyl ethyl amine, anacylin, sesamine, pellitorine, alkyl amides, lignans, tannins, resin and essential oil[1]. Despite a long tradition of use of *A. pyrethrum* for the treatment of various ailments, no

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pharmacological work has ever been carried out to prove its traditional claims. The exhaustive survey of literature suggested that the plant under investigations has been scientifically used in clinical study for erectile dysfunction and premature ejaculation[2] but the plant has never been investigated for their monographic study till date.

Keeping in view the traditional, alternative and complementary medicinal uses, sporadic phytochemical and pharmacological reports, *A. pyrethrum* seems to hold great potential for in-depth phytochemical studies and investigation for various biological activities. Therefore, it was considered worthwhile to undertake phytochemical and biological studies on this traditionally used and clinically potential plant and implement the following plan of work to achieve the goal.

MATERIALS AND METHODS

Plant material

A. pyrethrum Linn roots (Family - *Asteraceae*) were collected from the market in Barnala (Punjab) in the month of October 2011. The plant materials were authenticated by Dr. Avneet Pal Singh, Assistant Professor, Department of Botany, Punjabi University, Patiala. The specimen of plant has been deposited in the Department of Botany, Punjabi University, Patiala.

Chemical and instrumental materials

The AR / LR grade of various solvents, chemicals and reagents were used in present analytical studies and procured from E. Merck, New Delhi, India. Microscope, tray drier, solvent collector, watch glass, digital camera, sieve No. 250, sieve No. 100, ash less filter paper, Gooch crucible, single pan balance, muffle furnace, desiccators , reflux condenser, filter paper, Soxhlet apparatus, hot air oven, auto clave and micro pipette (Popular Traders, Ambala, Haryana, India) were employed.

Methods

The various extracts and fractions were prepared from plant roots as per various standard procedures reported in the literature[3]. Various physico-chemical parameters such as total ash, water soluble ash, acid insoluble ash, sulphated ash, petroleum ether soluble extractive, alcohol soluble extractive, water soluble extractive values, foreign organic matter, moisture content, fibre content, transverse section, powder microscopy, TLC fingerprinting profile and phytochemical screening were performed using standard procedures[4-9]. The total phenol content and antioxidant activity of plant samples were evaluated as per standard procedures. The antimicrobial profile of various extracts and fraction was performed using well known cup plate test[10].

RESULTS AND DISCUSSION

In recent years, ethno-medicinal studies received much attention on natural resources to throw light on the numerous medicines[5-6], especially of plant origin which needs evaluation on modern scientific lines such as phytochemical analysis, antimicrobial and antioxidant profile. The various pharmacognostic standards were established by using roots of the plant and each pharmacognostic standard experiment was performed in triplicate. The various pharmacognostic standards were established using the well-established procedures mentioned in Indian Pharmacopeia. The result of analysis of various physico-chemical parameters of the plant are presented in Table I.

Table I: Physicochemical parameter of *A. pyrethrum* root parts

Parameter	Observation (%w/w)
Total ash	8.5 %
Water soluble ash	5.2 %
Acid insoluble ash	6.7 %
Sulphated ash	8.2 %
Foreign organic matter	Nil
Moisture content	11 %
Fibre content	9.9 %
Petroleum ether soluble extractive value	2.3 %
Alcohol soluble extractive value	11.2 %
Water soluble extractive value	7.4 %

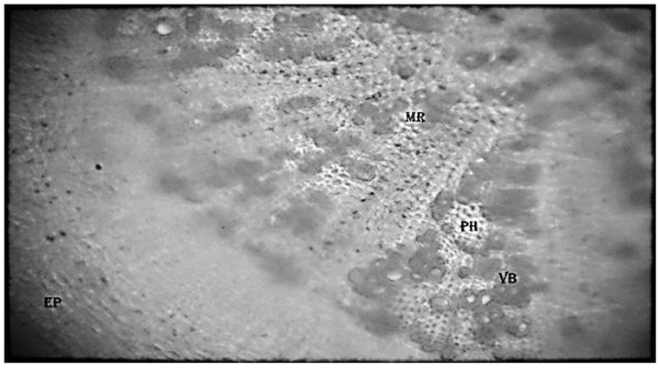

Fig. 1: Transverse section of *A. pyrethrum* root. EP-epidermis; PH- phloem; VB- vascular bundles and MR- medullary rays

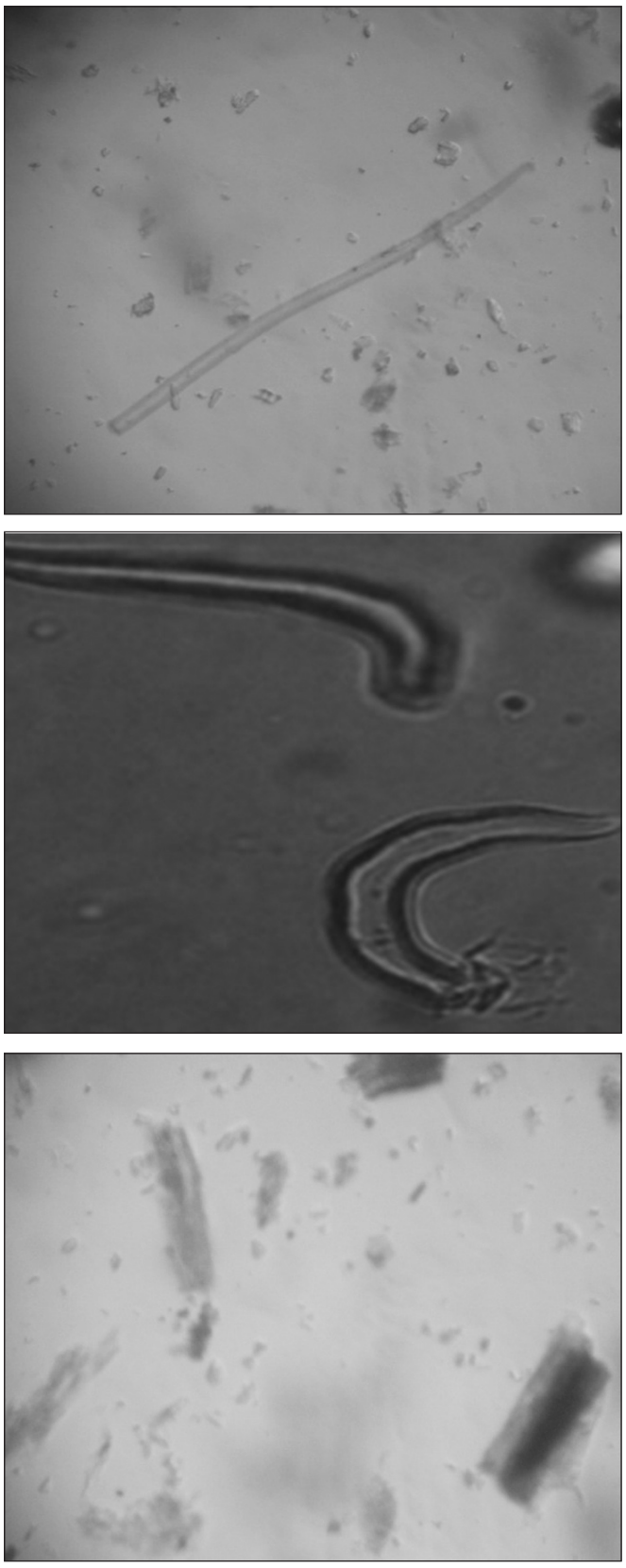

Fig. 2: Powder microscopic photomicrograph *A. pyrethrum* root. A-pericyclic fibre; B-unicellular trichomes and C-calcium oxalate crystals

The transverse section of the root of the plant showed the presence of epidermis, phloem, vascular bundles and medullary rays (Fig. 1). Pericyclic fibre, unicellular trichomes and calcium oxalate crystals were found in the powder microscopic studies (Fig. 2).

Amongst various chromatographic techniques, thin layer chromatography is a handy technique for studying separation pattern of various extracts of plant material[5-6]. TLC fingerprint profiles are useful for identification/ authentication of plant and material. In order to prepare qualitative TLC fingerprint profiles of petroleum ether, chloroform and methanol extracts of *A. pyrethrum* root parts, the plant material was subjected to a standardized extraction procedure to prepare various extracts. The dried extracts of petroleum ether, chloroform and methanol were dissolved in 3 mL of respective solvents, and their volume was made up to 5 mL in volumetric flasks. On TLC plates, 10 µL of the standard solution of each extract was loaded using 2 µL calibrated capillaries. Various mobile phases were used to get optimum resolution of compounds in various crude extracts. The thin layer chromatograms were visualized by spraying with 1 % anisaldehyde followed by heating at 105 ^{0}C for 2 minutes. The results of TLC fingerprint profile of various crude extracts are presented in Table II.

Table II: Results of thin layer chromatography of various extract plant roots

Extract	Mobile phase	Number of spots*
Petroleum ether	Hexane : chloroform	Three spots Rf values - 0.84,0.92,0.94
Chloroform	Toluene: ethyl acetate : glacial acetic acid	Seven spots Rf values - 0.19, 0.25, 0.32, 0.40, 0.42, 0.50, 0.62,
Methanol	Toluene: ethyl acetate : glacial acetic acid	Five spots Rf values - 0.48,0.53, 0.73,0.80, 0.88

**Spots were visualized by spraying with 1 % anisaldehyde followed by heating at 105 ^{0}C for 2 minutes.*

The various extracts of plant roots were successively prepared using Soxhlet extraction method. The %age yield of petroleum ether, chloroform, methanol and water extracts were found to be 5.5, 7.0, 6.5 and 5.0 % w/w, respectively. The phytochemical evaluation of various extracts showed the presence of sterols in

Table III: Antibacterial activity of various extracts and fraction of plant roots against Gram positive organism *S. aureus* MTCC 7443

Tested concentration / Dose (mcg)	Zone of inhibition (mm)				
	Petroleum ether extract	Chloroform extract	Methanol extract	Water extract	Ethyl acetate fraction
1000	6.43	8.83	10.72	6.82	12.84
2000	6.76	9.20	11.00	7.54	12.44
4000	7.34	9.76	10.36	8.17	11.22
8000	7.46	10.10	10.48	8.58	14.33
10 (Ciprofloxacin)	17.39	17.39	17.39	17.39	17.39

Table IV: Antibacterial activity of various extracts and fraction of plant roots against Gram positive organism *S. epidermides* MTCC 1133

Tested concentration / Dose (mcg)	Zone of inhibition (mm)				
	Petroleum ether extract	Chloroform extract	Methanol extract	Water extract	Ethyl acetate fraction
1000	7.16	6.62	8.62	7.19	11.02
2000	7.74	6.75	9.75	7.74	12.13
4000	7.81	6.84	9.83	7.81	13.65
8000	7.94	6.95	10.92	7.94	13.99
10 (Ciprofloxacin)	17.23	17.23	17.23	17.23	17.23

Table V: Antibacterial activity of various extracts and fraction of plant roots against Gram negative organism *P. aeruginosa* MTCC 2449

Tested concentration / Dose (mcg)	Zone of inhibition (mm)				
	Petroleum ether extract	Chloroform extract	Methanol extract	Water extract	Ethyl acetate fraction
1000	8.58	9.65	10.64	9.67	11.12
2000	9.46	9.92	11.10	9.77	14.54
4000	9.52	10.36	10.68	9.66	16.92
8000	9.86	10.62	11.16	10.22	17.38
10 (Ciprofloxacin)	28.18	28.18	28.18	28.18	28.18

Table VI: Antibacterial activity of various extracts and fraction of plant roots against Gram negative organism *E. coli* MTCC 1235

Tested concentration / Dose (mcg)	Zone of inhibition (mm)				
	Petroleum ether extract	Chloroform extract	Methanol extract	Water extract	Ethyl acetate fraction
1000	7.22	9.58	14.33	8.23	19.35
2000	8.21	10.55	13.37	9.21	19.57
4000	9.16	12.44	15.69	9.65	21.70
8000	9.55	13.58	16.17	10.81	22.70
10 (Ciprofloxacin)	29.71	29.71	29.71	29.71	29.71

Table VII: Antifungal activity of various extracts and fraction of plant roots against fungal strain *C. albicans* MTCC 1637

Tested concentration / Dose (mcg)	Zone of inhibition (mm)				
	Petroleum ether extract	Chloroform extract	Methanol extract	Water extract	Ethyl acetate fraction
1000	8.92	8.45	9.52	6.78	11.48
2000	9.34	8.78	9.69	7.44	12.12
4000	8.13	9.79	9.81	8.54	12.86
8000	8.87	9.88	10.29	9.27	13.23
10 (Fluconazole)	12.04	12.04	12.04	12.04	12.04

Table VIII: Antifungal activity of various extracts and fraction of plant roots against fungal strain *A. niger* MTCC 1235

Tested concentration / Dose (mcg)	Zone of inhibition (mm)				
	Petroleum ether extract	Chloroform extract	Methanol extract	Water extract	Ethyl acetate fraction
1000	6.32	6.0	8.45	6.54	10.54
2000	6.48	6.03	9.53	6.62	11.45
4000	6.72	6.59	9.67	6.83	11.85
8000	6.84	9.53	10.84	6.98	11.27
10 (Fluconazole)	10.34	10.34	10.34	10.34	10.34

Table IX: Antioxidant activity of various test samples of plant roots

Treatment	Antioxidant activity					
Rutin	Conc.	2	4	6	8	10
	Mean % age	41.00±0.82	74.77±1.05	76.64±0.57	77.19±0.38	77.52±0.19
Petroleum ether	Conc.	10	20	40	60	80
	Mean % age	10.16±0.55	16.12±0.35	15.52±0.34	20.50±0.29	20.81±087
Chloroform	Conc.	10	20	40	60	80
	Mean %a ge	11.58±0.43	13.62±0.34	25.62±0.29	43.21±0.27	48.41±0.11
Methanol	Conc.	10	20	40	60	80
	Mean % age	50.39±0.13	67.26±0.17	83.39±0.29	87.29±0.36	91.46±0.29
Water	Conc.	10	20	40	60	80
	Mean % age	10.41±0.18	13.10±0.26	15.44±0.23	16.22±0.25	18.7±0.12
Ethyl acetate	Conc.	10	20	40	60	80
	Mean % age	90.41±0.22	93.68±0.16	93.77±0.22	93.92±0.21	94.34±0.17

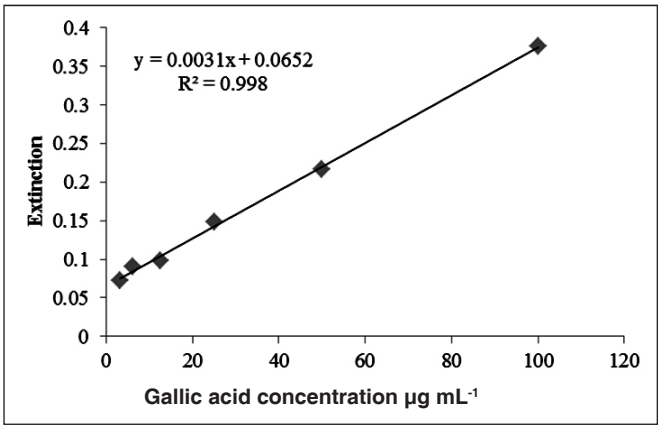

Fig. 3: Standard curve of extinction against gallic acid concentration

petroleum ether extract, alkaloids in chloroform extract, anthraquinone glycoside, flavonoids, tannins and carbohydrates in methanol extract and carbohydrates, protein and anthraquinone glycoside in water extract. The flavonoid rich fraction / ethyl acetate rich fraction was prepared from methanol extract by reflux method and %age yield of fraction was found to be 35.2 % w/w in relation to methanol extract. The ethyl acetate fraction was showing presence of flavonoids and tannins as major classes of compounds.

Total phenols were determined spectrophotometrically and percentage was recorded. The standard curve was obtained by using different concentration of gallic acid between 6.25 to 100 µL mL^{-1} (Fig. 3). The highest percentage of polyphenols at 6.48 % was found in ethyl acetate fraction, whereas methanol extract was found to possess only 2.40 % of total phenols. This observation suggests that most of phenols in the methanol extract could be isolated using ethyl acetate.

In antimicrobial studies, various extracts viz., pet ether, chloroform, methanol, water extracts and ethyl acetate fraction of methanol extract were tested against Gram positive organisms (*S. aureus* MTCC 7443 and *S. epidermides* MTCC 1133), Gram negative organisms (*P. aeruginosa* MTCC 2449 and *E. coli* MTCC 1235) and fungal strains (*C. albicans* MTCC 1637 and *A. niger* MTCC 1235). The zone of inhibition in mm was determined by cup plate method. More the zone of inhibition, more the antimicrobial activity of the extracts/fraction.

In antibacterial study, the ethyl acetate fraction exhibited maximum antibacterial activity against *S. aureus* MTCC 7443, *S. epidermides* MTCC 1133, *P. aeruginosa* MTCC 2449 and *E. coli* MTCC 1235, followed by methanol extract. The other extracts were found to be devoid of antibacterial activity. The range of the zone of inhibition

was 6.62 to 29.71 mm. The results of antibacterial profile of test samples are presented in Tables III-VI.

In antifungal study, ethyl acetate fraction exhibited maximum antibacterial and antifungal activities against *C. albicans* MTCC 1637 and *A. niger* MTCC 1235, followed by methanol extract. The other extracts were found to be devoid of antifungal activity. The range of the zone of inhibition was 6 to 13.22 mm. The results of antifungal profile of test samples are presented in Tables VII-VIII.

Antioxidants help to reduce the incidence of degenerative diseases such as arthritis, arteriosclerosis, cancer, heart disease, inflammation and brain dysfunction. In addition, antioxidants have been reported to retard ageing besides preventing or delaying oxidative damage of lipids, proteins and nucleic acids caused by reactive oxygen species. Among the most abundant antioxidants in fruits are polyphenols and ascorbic acid. The phytochemical evaluation revealed the presence of phenols in plant roots. So it was decided to evaluate different extracts and fraction for antioxidant activity using DDPH assay at 517 nm[9]. The DPPH assay measures the ability of the extracts or fraction to donate hydrogen to the DPPH radical resulting in bleaching of DPPH solution. The higher antioxidant activity is related to higher bleaching action. The petroleum ether, chloroform and water extracts showed negligible antioxidant activity whereas methanol extract and its ethyl acetate fraction showed significant activity. Ethyl acetate fraction showed maximum activity with percentage inhibition of 90.41 at concentration of 10 µg mL^{-1}, followed by methanol extract of 50.39 at concentration of 10 µg mL^{-1}. A slight increase in antioxidant activity was observed in methanol extract as well as ethyl acetate fraction in concentration dependent manner. So, it is clear that phenols are responsible for antioxidant activity of medicinal and herbal drugs. The results of antioxidant profile of test samples are presented in Table IX.

CONCLUSION

It can be concluded that polyphenols are responsible for the biological activities of *A. pyrethrum* roots. In future, we propose to isolate polyphenols and investigate the isolated polyphenols for their detailed biological activities by different methods, so that the the plant may be established as potent anti-microbial and antioxidant drug.

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