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ANTIOXIDANT ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *ANACYCLUS PYRETHRUM*

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ABSTRACT

In present study hydroalcoholic extract of *Anacyclus pyrethrum* was investigated for the phytochemical screening and antioxidant activity. Phytochemical screening of the plant showed the presence of glycosides, carbohydrates, phenols, alkaloids, flavonoids, proteins and amino acids and diterpenes. Antioxidant activity was assessed by using 2, 2- diphenyl-1-picryl-hydrazyl (DPPH) assay using ascorbic acid as standard antioxidant. The data obtained from all these models clearly suggested that the antioxidant activity of hydroalcoholic extract of *Anacyclus pyrethrum* was significant and dose dependent. The present study reveals that hydroalcoholic extract of *Anacyclus pyrethrum* can be used as a potential source of natural antioxidant which may be treat various oxidative stress related diseases.

Key words: *Anacyclus pyrethrum*, Hydroalcoholic extract, Phytochemical screening, Antioxidant activity.

INTRODUCTION:

The interaction between free radicals, antioxidants, and co-factors is essential in maintaining health, aging and age-related neurodegenerative diseases (Agarwal and Sohal. 1996). Free radical induces oxidative stress. Imbalance between oxidants and antioxidants causes oxidative stress. Hence, for maintaining a good biological system the equilibrium between free radicals and antioxidants is believed to be a critical concept. Even though, our biological systems has an internal defense mechanism to fight against intracellular free radicals at a certain point this fails due to the over expression of free radicals (Halliwell and Gutteridge, 1986). Hence, identification of alternate source of antioxidants is required for conferring protection to body cells. Literature survey shows the significant role of reactive oxygen species and other oxidants in causing numerous disorders and diseases (Pramod et al. 2013; Shinde et al., 2012; Kunwar and Priyadarsini, 2011). This has gained the attention of scientists to an appreciation of antioxidants helping in the maintenance of human health for prevention and treatment of diseases.

Antioxidant plays a key role in preventing and scavenging free radicals providing safeguard

to humans against diseases. Recent research has been directed towards "Natural antioxidants" from the herbal plants due to safe therapeutic potential. In the developing countries more interest in medicinal plants arises from their long use in folk medicines as well as their prophylactic properties. Previous research shows the inverse relation between the mortality from diseases and the consumption of plant products, which could be due to the presence of various antioxidant compounds (Scalbert et al., 2005; Spencer et al., 2007; Beckman, 2000). Recently there has been an upsurge of interest in the therapeutic potential medicinal plants as antioxidants in reducing oxidative stress. Among the numerous naturally occurring antioxidants; ascorbic acid, carotenoids and phenolic compounds are more effective in inhibiting lipid peroxidation to scavenge free radicals and active oxygen species.

Anacyclus pyrethrum DC roots and leaf have important role in the traditional Ayurvedic and Unani system of holistic health and herbal medicine of the East. Especially the root of *Anacyclus pyrethrum* is reported to have good medicinal values in traditional system of medicine (Kishor and Lalitha, 2012). *Anacyclus pyrethrum* from Asteraceae family and Anacyclus genus is a native plant of India and Arabic countries and its root has therapeutic effects (Naderi et al., 2012). *Anacyclus pyrethrum* (Linn) De Candolle, commonly known as 'Spanish pyrethrum root' in English, 'Aaqarqarhaa' in Unani, and 'Aaqarqarhaa' in Ayurveda. It is widely recognized in Ayurvedic system of Indian medicine as tonic and rejuvenator. Its root is hard, compact, fusi-form about the size of the little finger, with sometimes leaf - remnants at the top, and beset with few or no hair-like rootlets; externally brownish, deeply fissured longitudinally (Puri, 2003). It contains essential oils and an alkaloid pellitorine that is intensely pungent constituent with a mixture of isobutyl amide. Traditionally, plant is used as antibacterial, anti-inflammatory and tonic to the nervous system (Tyagi et al., 2011). *Anacyclus pyrethrum* commonly known as pellitory and Akarkara in Hindi local language is perfectly recognized in traditional and herbal medicine and has a positive effect on regulating the immune system (Sharma et al., 2010). Therefore, the present study consists of investigation of the antioxidant potential of hydroalcoholic extract of *Anacyclus pyrethrum* and to put forward the evidence of the fact that this plant has immense therapeutic power. This is the first report regarding antioxidant activity and phytochemicals study of the leaves of this plant.

MATERIALS AND METHODS

Plant material collection

The plant *Anacyclus pyrethrum* was collected from local area of Bhopal (M.P.) in the month of Jan, 2018.

Storage

Drying of fresh aerial parts was carried out in sun but under the shade. Dried *Anacyclus pyrethrum* was preserved in plastic bags and closed tightly and powdered as per the requirements.

Defatting and extraction of plant material

Anacyclus pyrethrum was shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place. Dried

powdered *Anacyclus pyrethrum* has been extracted with hydroalcoholic solvent using maceration process for 48 hrs, filtered and dried using vaccum evaporator at 40 ºC.

Phytochemical screening:

Phytochemical examinations were carried out for all the extracts as per the standard methods (Kokate, 1994; Harborne, 1973).

***In-vitro* free radical scavenging activity (2, 2-diphenyl-1-picrylhydrazyl - DPPH)**

The DPPH radical scavenging activity of all the extracts was evaluated by the method described by Lee JY *et al.,* 2004. with slight modification. Ascorbic acid (10-100 µg/ml) was used as the standard. Plant extract (1.5 ml) at different concentrations (10-100 µg/ml) were treated with 1.5 ml of 0.2 mmol DPPH (2,2-diphenyl-1-picrylhydrazyl) in ethanol solution. The reaction mixture was incubated in the dark at room temperature for 30 min. The absorbance of the sample and standards was measured at 517 nm. The ability of the plant extract and standard to scavenge the DPPH radical was calculated as percentage inhibition of absorbance by using the following formula and IC_{50} values were determined.

Calculation of % reduction =

$$\frac{\text{Control Absorbance - Test absorbance}}{\text{Control Absorbance}} \times 100$$

Results and discussion

Phytochemical screening of the plant showed the presence of glycosides, carbohydrates, phenols, alkaloids, flavonoids, proteins and amino acids and diterpenes. Many flavonoids are found to be strong antioxidants effectively scavenging the reactive oxygen species because of their phenolics hydroxyl groups (Balasundram et al., 2006). Phenolic antioxidants are generally believed to form phenoxyl radical upon donating a hydrogen atom that could quench active free radicals. This has been reported to have multiple biological effects (Cao et al., 2009).

Table 1: Phytochemical screening of hydroalcoholic extract of *Anacyclus pyrethrum*

S. No.	Test	*Anacyclus pyrethrum*
1.	Detection of alkaloids: a) Hager's Test: b) Dragendroff's Test:	 -ve -ve
2.	Detection of carbohydrates: a) Fehling's Test:	 +ve
3.	Detection of glycosides: a) Legal's Test:	 +ve
4.	Detection of saponins a) Froth Test:	 -ve
5.	Detection of phenols a) Ferric Chloride Test:	 +ve
6.	Detection of flavonoids a) Alkaline Reagent Test: b) Lead acetate Test:	 +ve +ve
7.	Detection of proteins and aminoacids a) Xanthoproteic Test:	 +ve
8.	Detection of diterpenes a) Copper acetate Test:	 +ve

+ve = present -ve = absent

Antioxidant activity of the antioxidants is concerning with those compounds capable of protecting the organism system against the potential harmful effect of oxidative stress. In this study, the antioxidant capacity of hydroalcoholic extract of *Anacyclus pyrethrum* was accessed by DPPH scavenging activity. IC_{50} of DPPH scavenging activity of hydroalcoholic extract was compared to IC_{50} of ascorbic acid.

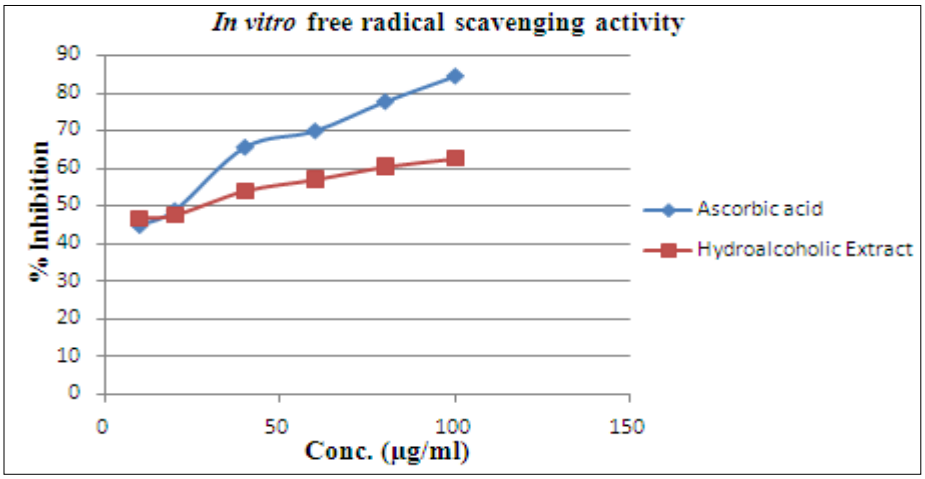

Figure 1: Graph of *in vitro* free radical scavenging activity

Table 2: Result of *in vitro* free radical scavenging activity

S. No	Ascorbic acid			*Anacyclus pyrethrum*		
	Conc. (µg/ml)	Test	% Inhibition	Conc. (µg/ml)	Test	% Inhibition
1	10	0.321	44.65517	10	0.124	46.55172
2	20	0.298	48.62069	20	0.122	47.41379
3	40	0.201	65.34483	40	0.107	53.87931
4	60	0.176	69.65517	60	0.100	56.89655
5	80	0.131	77.41379	80	0.092	60.34482
6	100	0.092	84.13793	100	0.087	62.5
IC_{50} (µg/ml)			24.18		IC_{50}	27.15

The DPPH method was evidently introduced nearly 50 years ago and it is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant capacity. The parameter IC50, is used for the interpretation of the results from the DPPH method and is defined as the concentration of substrate that causes

50% loss of the DPPH activity. Table 2 showed the DPPH radical scavenging activity of hydroalcoholic extract of *Anacyclus pyrethrum*. DPPH is a stable free radical which is reduced in the presence of hydrogen donating antioxidants. The scavenging ability of hydroalcoholic extract of *Anacyclus pyrethrum* for free radicals of 1, 1-diphenyl 1-2-picrylhydrazyl (DPPH) showed remarkable scavenging activities. Hydroalcoholic extract showed the highest scavenging activity (lowest IC50; 27.15 µg/ml) followed by ascorbic (IC50; 24.18 µg/ml). These scavenging properties are generally due to high reducing capacity of the polyphenols acting as primary antioxidants (Odabasoglu et al., 2004). 1, 1-Diphenyl-2-pecryl-hydrazyl (DPPH) is a stable free radical which has an unpaired valence electron at one atom of nitrogen bridge (Eklund et al., 2005).

Conclusion

The phytochemical screening of hydroalcoholic extract of *Anacyclus pyrethrum* showed the presence of glycosides, carbohydrates, phenols, alkaloids, flavonoids, proteins and amino acids and diterpenes. The study for their anti-oxidant potential has provided substantial positive data pointing towards the evidence of antioxidant activity. The data obtained from in vitro free radical scavenging activity (2, 2-diphenyl-1-picrylhydrazyl - DPPH) assay clearly suggested that the antioxidant activity of hydroalcoholic extract of *Anacyclus pyrethrum* was dose dependent. So these findings of present study suggest that this plant is a potential source of natural antioxidant. Further studies are warranted for the isolation and characterization of antioxidant compounds, and also in vivo studies are needed for understanding their mechanism of action as antioxidants.

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