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PHYTOCHEMICAL ANALYSIS AND IN VITRO ANTIOXIDANT ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *ANACYCLUS PYRETHRUM*

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ABSTRACT

In this investigation, the antioxidant capacity of *Anacyclus pyrethrum* was assessed. The plant material was collected, extracted & subjected for qualitative & quantitative study. Through the use of DPPH free radical, nitric oxide radical inhibition, Hydrogen peroxide method, antioxidant activity was additionally, the amounts of total phenolics, flavonoids, and proanthocyanidin were measured. Results showed that the percentage loss of *Anacyclus pyrethrum* was found to be 17.8%. The % yield was observed to be 1.65% and 5.21% in pet ether & hydroalcoholic extract respectively. The flavonoids, saponins, proteins, carbohydrate, phenol, alkaloids and diterpenes were present in hydroalcoholic extract of *Anacyclus pyrethrum*. The total phenol & flavonoid content was observed to be 0.397 and 0.733 mg/100mg respectively. The IC_{50} value for hydroalcoholic extract was seen to be 67.78 for DPPH assay, the standard ascorbic acid IC_{50} value was estimated to be 18.69. While the IC_{50} value obtained by NO method is 87.33% for the extract & IC_{50} value of ascorbic acid in this case is 18.69%. Further, by hydrogen peroxide method the IC_{50} value obtained for extract was 88.03% with comparison to IC_{50} value of 36.75 for ascorbic acid. Thus, Studies have shown that *Anacyclus pyrethrum* extracts have the ability to scavenge free radicals, reduce lipid peroxidation, and scavenge superoxide radicals. This suggests that *Anacyclus pyrethrum* may have antioxidant activity, which could be beneficial in treating oxidative stress-related diseases.

Keywords: Oxidative stress, Medicinal plants, Antioxidants, *Anacyclus pyrethrum*, Phenol, Flavonoid.

INTRODUCTION

Oxidative stress plays a crucial role in the development and progression of cancer, diabetes mellitus, cardiovascular disease, neurodegenerative illness, and inflammatory disease. The situation is caused by the cell's ineffective quenching of free oxygen and nitrogen species or excessive production of these species. Free oxygen and nitrogen species are unstable molecules that are produced both endogenously (in the body) and exogenously (in the environment) as a result of the body's typical aerobic metabolic processes. Cigarette smoke, exposure to ozone, ionizing radiation like X-rays, and medications are only a few examples of exogenous sources of free radicals. As opposed to this, endogenous sources of free radicals include the xanthine oxidase pathway and electron transfer chain processes in the mitochondria during disease states such inflammation, ischemia, and reperfusion injury (Arika *et al*., 2019; Moriasi *et al*., 2020; Bhat *et al*., 2015).

The endogenous anti-oxidative defense mechanism balances the formation of reactive oxygen species (ROS) under normal physiological conditions. Enzymes including glutathione reductase, catalase, superoxide dismutase, and glutathione peroxidase are the main types of endogenous antioxidants. Contrarily, non-enzymatic endogenous antioxidants produced by the body's metabolism include glutathione and lipoic acid. Enzymatic antioxidants, which provide the initial line of defense, change reactive superoxide and hydrogen peroxide into water and oxygen. By quickly neutralizing radicals and oxidants, non-enzymatic antioxidants can serve as a second line of defense against ROS. The third line of defense in the detoxification and elimination process is the enzymatic antioxidants. Exogenous antioxidants with in vivo activity include dietary antioxidants such vitamins, carotenoids, polyphenols, flavonoids, and bioflavonoids (Mironczuk-Chodakowska *et al*., 2018; Aguilar, 2016).

Natural antioxidants derived from plant extracts or isolated compounds of plant origin are increasingly replacing synthetic antioxidants, which raise safety concerns. Numerous organic substances with antioxidant properties can be found in plants. These substances can be divided into three classes: terpenoid groups, polyphenols (flavonoids, phenolic acids, stilbenes, and lignans), and vitamins (Vitamin C and E). Vitamin C and E are abundant in fruits and vegetables. Rosaceae (sour cherry, strawberry, blackberry), Empetraceae (cowberry), Ticaceae (blueberry), Asteraceae (sunflower seed), and Punicaceae (pomegranate) are some of the fruit families that are particularly high in these vitamins. Vegetable groups with high vitamin C and E content include broccoli, Brussels sprouts, green cabbage, tomatoes, cauliflower, lettuce, and leeks. Plant vitamins serve as the main sources of antioxidants. Vitamin E serves as a crucial lipid-soluble antioxidant, and vitamin C guards against cellular damage brought on by oxidative stress. Both vitamin E and vitamin C are utilized in foods as antioxidants, however vitamin C's impact is minimal (Fierascu, *et al*., 2018; Kotha *et al*., 2022).

The antioxidant capacities of phytochemicals derived from plants have been assessed using a variety of analytical techniques. The chemical structure of the substance affects its antioxidant activity, specifically its capacity to donate hydrogen with an electron, metal chelation, and ability to delocalize the unpaired electron within the aromatic structure. Anacyclus pyrethrum's potential as an antioxidant will therefore be the focus of this investigation.

The plant *Anacyclus pyrethrum*, sometimes referred to as akarakara, is a member of the Asteraceae family. The plant contains aphrodisiac and antioxidant potentials, antidiabetic, insecticidal, and immune-stimulating effects, among other significant medical characteristics. Previous studies have also documented other conventional uses, including those for rheumatism, sciatica, colds, neuralgia, and paralysis. This species is regarded as a sialagogue and is used to treat stomach disorders, diseases of the mouth that cause inflammation, cysts in the genital tract, and toothaches. Therefore, more investigation is required to see if there is any connection between phytochemistry, toxicity, and traditional usage. Additionally, A. pyrethrum is extremely promising as a drug, so more

clinical studies should be carried out to demonstrate its effectiveness (Jawhari *et al*., 2020; Kushwaha *et al*., 2012).

MATERIALS & METHODS

Collection of plant material

Fresh leaves of *Anacyclus pyrethrum* were collected from local area of Bhopal (M.P.) in the month of May, 2022.

Drying

The leaves of *Anacyclus pyrethrum* were separated and washed with sterile distilled water to remove the adhering dust particles and other unwanted materials. Drying of fresh plant parts were carried out in sun but under the shade.

Percentage loss

The % loss was calculated by dividing loss in weight of sample by weight of sample multiplied by 100.

Defatting of Plant Material

55.89 gram of dried leaves of *Anacyclus pyrethrum* was coarsely powdered and subjected to extraction with petroleum ether (60-80°C) in a maceration method**.**

Extraction procedure

Defatted dried leaves of *Anacyclus pyrethrum* were extracted with hydroalcoholic solvents (Ethanol: water; 75:25v/v) by maceration method.

Determination of percentage yield

The % yield was calculated by dividing weight of extract by weight of powder taken multiplied by 100.

Phytochemical screening

Phytochemical examinations were carried out for all the extracts as per the standard methods.

Total phenolic content estimation

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10-50μg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer (Neupane and Lamichhane, 2020).

Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride method. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 10-50μg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids. 1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm (Chang *et al*., 2002).

In-vitro antioxidant activity using different methods

DPPH method

Total free radical scavenging capacity of the hydroalcoholic extracts from leaves of *Anacyclus pyrethrum* were estimated according to the previously reported method with slight modification (Parkhe and Bharti, 2019). Solution of DPPH (6 mg in 100ml methanol) was prepared and stored in dark place. Different concentration of standard and test (10- 100 µg/ml) was prepared. 1.5 ml of DPPH and 1.5 ml of each standard and test was taken in seperate test tube; absorbance of this solution was taken immediately at 517nm. 1.5 ml of DPPH and 1.5 ml of the methanol was taken as control absorbance at 517nm.

Nitric oxide method

Nitric oxide was produced from sodium nitroprusside and the Griess reagent was measured. Sodium nitroprusside spontaneously produces nitric oxide in aqueous solution at physiological pH, interacting with oxygen to generate nitric ions that can be estimated using Griess reagent. Nitric oxide scavengers compete with oxygen resulting in decreased nitric oxide manufacturing (Sueishi *et al.,* 2011). Sodium nitroprusside (10 mmol / L) was mixed with various extract concentrations in phosphate buffer saline (PBS) and incubated at 25°C for 150 min. Griess reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% napthylethylenediamine dihydrochloride) was added to the specimens. The chromophore absorbance created during the diazotization of sulphanilamide nitrite and subsequent coupling with napthylethyleneediamine was read at 546 nm and referred to the absorption of conventional ascorbic acid solutions treated in the same manner with Griess reagent as a positive control.

Hydrogen peroxide method

In-vitro antioxidant activity of extract of leaves of *Anacyclus pyrethrum* using hydrogen peroxide was performed as Czochra and Widensk (2002) proposed. Added 2ml hydrogen peroxide (43 mol) and 1.0 ml hydroalcoholic sample [20-100 µl different extracts (4 mg / ml) ethanol] accompanied by 2.4 ml 0.1 M phosphate buffer (pH 7.4). The resulting solution was maintained for 10 minutes and the absorbance at 230 nm was recorded. Without adding hydrogen peroxide, blank was ready and control was prepared without sample. It was used as a conventional compound with ascorbic acid. Free radical hydrogen peroxide scavenging activity (percent) has been calculated .

RESULTS AND DISCUSSION

The Percentage loss of *Anacyclus pyrethrum* was found to be 17.8%. The % yield was observed to be 1.65% and 5.21% in pet ether & hydroalcoholic extract respectively. The flavonoids, saponins, proteins, carbohydrate, phenol, alkaloids and diterpenes were present in hydroalcoholic extract of *Anacyclus pyrethrum*. The total phenol & flavonoid content was observed to be 0.397 and 0.733 mg/100mg respectively. The IC 50 value for hydroalcoholic extract was seen to be 67.78 for DPPH assay, the standard ascorbic acid IC 50 value was estimated to be 18.69. While the IC 50 value obtained by NO method is 87.33% for the extract & IC 50 value of ascorbic acid in this case is 18.69%. Further, by hydrogen peroxide method the IC 50 value obtained for extract was 88.03% with

comparison to IC50 value of 36.75 for ascorbic acid.

According to findings reported on plant extracts of *Anacyclus pyrethrum* the hydrogen peroxide scavenging activity of the examined plant extracts in this investigation decreased with concentration. On the other hand, other studies have found that hydroxyl radical scavenging activities have increased in a concentration-dependent manner. The saturation of the reactive centers of hydroxyl radicals by high extract concentrations leading to low activities, as opposed to dilute concentrations that ensured easier and faster reaction, leading to high activations, could have caused the concentration-dependent decrease in hydroxyl radical scavenging activity.

These phytochemicals' reductive and oxidative abilities, which permit the absorption of free radicals and the reversal of their effects, are assumed to be the source of their antioxidant capacity. There are fewer cases of death and suffering from illnesses linked to oxidative stress since many of these secondary metabolites have strong reductive properties. Due to their biological impacts, our work strongly implies that phenolics are important parts of these plants.

According to the data, the extract of plant *Anacyclus pyrethrum* exhibit significant antioxidant activity. They also include phytochemicals that are linked to antioxidants. It is advised that more research be done with the goal of extracting and defining the pure phytoactive components. To determine the safety of the extracts of *Anacyclus pyrethrum* toxicity tests should be conducted.

Table 1: Showing the results of percentage loss of *Anacyclus pyrethrum*

S. No.	Description	Weight in (gms.)	% Loss
1.	Weight of plant material in wet, fresh condition	73	
2.	Weight of plant material after drying at room temperature	60	17.8%
3.	Loss in weight on drying	73-60=13	

Table 2: Results of percentage yield of *Anacyclus pyrethrum*

Extracts	Percentage yield (%)
Pet. ether	1.65%
Hydroalcoholic	5.21%

Table 3: Result of phytochemical screening of hydroalcoholic extract of *Anacyclus pyrethrum*

S. No.	Constituents	Hydroalcoholic extract
1.	Alkaloids A) Wagner's Test: B) Hager's Test:	 +Ve -Ve
2.	Glycosides A) Legal's Test:	 -Ve
3.	Flavonoids A) Lead acetate Test: B) Alkaline Reagent Test:	 +Ve -Ve
4.	Saponins A) Froth Test:	 +Ve
5.	Phenol A) Ferric Chloride Test:	 +Ve
6.	Proteins A) Xanthoproteic Test:	 +Ve
7.	Carbohydrate A) Fehling's Test:	 +Ve
8.	Diterpenes A) Copper acetate Test:	 +Ve

Table 4: Estimation of total phenol and flavonoids content of of *Anacyclus pyrethrum*

S. No.	Total phenol content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1.	0.397	0.733

Table 5: % Inhibition of ascorbic acid and extract of *Anacyclus pyrethrum* using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Hydroalcoholic extract
1	10	30.42	18.63
2	20	59.11	24.87
3	40	67.48	39.42
4	60	75.25	51.54
5	80	77.58	56.02
6	100	79.63	62.14
IC 50		**18.69**	**67.78**

Table 6: % Inhibition of ascorbic acid and extract of *Anacyclus pyrethrum* using NO method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Hydroalcoholic extract
1	20	40.52	13.68
2	40	52.01	24.75
3	60	61.74	40.58
4	80	76.89	47.61
5	100	84.15	53.47
IC 50		**36.75**	**87.33**

Table 7: % Inhibition of ascorbic acid and extract of *Anacyclus pyrethrum* using hydrogen peroxide method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Hydroalcoholic extract
1	20	45.84	19.64
2	40	59.15	29.51
3	60	67.23	32.68
4	80	78.52	47.15
5	100	89.75	56.74
IC 50		**26.23**	**88.03**

CONCLUSION

Anacyclus pyrethrum is a medicinal plant found in the Mediterranean region and North Africa. It is used in traditional medicine to treat a variety of ailments, including inflammation, digestive issues, and skin disorders. Its active components are terpenoids, flavonoids, and polyphenols, which have been studied for their potential antioxidant activity. Studies have shown that *Anacyclus pyrethrum* extracts have the ability to scavenge free radicals, reduce lipid peroxidation, and scavenge superoxide radicals. This suggests that *Anacyclus pyrethrum* may have antioxidant activity, which could be beneficial in treating oxidative stress-related diseases.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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