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Research Article

DETERMINATION OF ANTI-ARTHRITIC ACTIVITY OF *NYCTANTHES ABORTRISTIS* FLOWER EXTRACT USING COMPLETE FREUND'S ADJUVANT (CFA)-INDUCED ARTHRITIS MODEL

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ABSTRACT

Nyctanthes abortristis belongs to the family Oleaceae having various medicinal values. Different parts of plants such as stem, bark, leaves and flowers have been reported already for their pharmacological properties. The present study is focused on the anti-arthritic potential of *Nyctanthes abortristis* flowers extracted from different solvents. Qualitative and quantitative estimation of isolated compounds and antioxidant activity has been studied using various antioxidant assays. Methanolic extract was used to determine the anti-arthritic activity in Complete Freund's adjuvant (CFA) induced arthritis rats by oral administration of crude extract at 200mg/kg and 400mg/kg dose concentrations. Paw volume analysis and haematological parameter were analysed on 28th day of treatment. The results exhibited significant anti-arthritic activity for *N. abortristis* flower extract.

Keywords: *Nyctanthes abortristis,* Anti-arthritic, Freund's adjuvant.

1. INTRODUCTION

Generally, arthritis is known as an inflammatory disease of joints that affects peoples of any age group. According to the WHO report (2003), prevalence of musculoskeletal disorders are most frequent cause of disability in the modern world in which Rheumatoid Arthritis [1] is epidemic autoimmune disorder by developing the chronic inflammation in joints of the body [2]. Rheumatoid arthritis affects to human life by severe debility, arrival comorbidities [3-7] and monetarily too [5]. About 1% of the world's population is afflicted by rheumatoid arthritis while women are more prone [7]. As rheumatoid arthritis is a chronic disorder, it needs an unceasing treatment for the affected individual [8]. The conventional medication known for this disorder is disease modifying anti-rheumatoid drugs (DMARDs), nonsteroidal anti-inflammatory drugs (NSAIDs), and corticosteroids [8]. Due to the adverse effect and failure to respond in about 30 % of the patients [9], people are very likely to seek alternative treatments to cure disease in order to complementary and alternative medicine (CAM). Researches indicate that 60-80 % of the arthritic patients rely on herbal therapies for the safety and efficacy [10].

Hence, the synthetic drugs have their own massive effects, so our medical world is turning towards the *Ayurvedic* science. Ethnobotany plays an important role to understand the relationship between plants and human in context of their vital role to maintain health and curing disease [11]. Medicinal plants contain various phytochemicals with significant properties. Phyto-chemicals produced definite functional activity against various diseases to prevent health of individuals and communities [12]. In manner of continuous search of new biological active compounds scientists studied the chemical constituents of different ornamental plants as well as for their medicinal values.

Nyctanthes arbor-tristis Linn (Nyctanthaceae) commonly called as night jasmine or coral jasmine is mainly characterized by the presence of phynylethanoid derivatives and iridoids glycosides [13]. It is a hardy large shrub or small tree widely distributed in outer Himalayan ranges from Chenab to Nepal, Assam, Burma, Bengal, Central India to Godavari, and in many parts of India. It has long been medicinally used in traditional systems of medicine [14]. Different parts of *Nyctanthes arbor-tristis* are known to own for dealing with various ailments such as leaves are used extensively for the treatment of various diseases such as malaria,

sciatica, chronic fever [15], rheumatism [16,17], internal worm infections [18] and also as a laxative, diaphoretic, diuretic [18]. Antitrypanosomal, anti-inflammatory and antioxidant were furthermore reported in stem bark and root extract of plant [19-21]. *Nyctanthes arbor-tristis* leaves [22-26] and seeds [27-30] contain number of iridoid glycosides hence it is widely used in the treatment of inflammatory disorder. In this consequence from various reviews reported, there is no any scientific validation for anti-arthritic activity, so the focus of present study is to determine the anti oxidant and anti-arthritic activity of *Nyctanthes arbor-tristis* flower extract.

2. MATERIAL AND METHODS

2.1. Sample Collection

Flowers of *Nycanthes arbotristis were* collected from Bhopal region (M.P.) and authenticated by Dr. Zia-Ul-Hasan, Head & Botonist, Department of Botany, Safia College of Science, Bhopal (M.P.) India.

2.2. Extraction & Photochemical Screening

The powdered plant materials were successively extracted initially with petroleum ether then ethyl acetate and methanol by continuous hot percolation method using soxhlet apparatus. Solvent was evaporated at 40°C to obtain yield of a solid mass. Preliminary phytochemical screening [31, 32], total phenolic content [33, 34] and total flavonoid content [35] were estimated using standard protocols with some modifications.

2.2.1. Total Phenol Content

TPC was estimated by Folin-Ciocalteu Reagent using Gallic acid as a standard. 0.5 ml of different concentrations of Gallic acid as well as test sample with 2 ml Folin-Ciocalteu reagent (1:10 in deionized water) and 4 ml of sodium carbonate solution was prepared. Later, this solution was incubated at room temperature for 30 min with intermittent shaking. Absorbance was recorded at 765 nm. Standard curve of Gallic acid was prepared.

2.2.2. Total Flavonoid Content

Diluted sample (0.5-Ml) was mixed with 2 mL of distilled water and subsequently with 0.15 mL of a 5% $NaNO_2$ solution. After 6 min, 0.15 mL of a 10% $AlCl_3$ solution was added and incubated for 6 min, then 2 mL of 4% NaOH solution was added to the stock. Immediately, the final volume was made upto 5 mL, allowed to stand for another 15 min. Absorbance was measured at 510 nm and standard curve was prepared for different concentration of Rutin.

2.3. *In-vitro* Antioxidant Activity of Extracts

2.3.1. DPPH Assay

The free radical scavenging activity of the extracts was determined *in vitro* by 2, 2- diphenyl-1-picrylhydrazyl (DPPH) assay [36, 37]. Methanolic solution (0.1 mM) of DPPH and test samples in 10-100 µg/mL concentration was prepared. 2 mL test sample was added in 1 ml of DPPH solution and incubated in dark for 10 mins. Absorbance was taken at 515 nm using double beam UV-Vis spectrophotometer and percent inhibition was determined.

2.3.2. Super oxide scavenging assay [38-39]

Reaction mixture was prepared containing 0.1 ml of Nitroblue Tetrazolium (NBT), 0.3 ml of extract and 1 ml of alkaline DMSO was added to produce a final volume of 1.4 ml and absorbance was measured at 560 nm against blank and reaction mixture without extract used as control.

2.3.3. Reducing Power assay

Phosphate buffer (0.5 ml) was added in 0.5ml sample of different concentrations with 0.5 ml of potassium ferricyanide and incubated for 20 mins at 50°C. 1.5 ml of trichloroacetic acid was added after cooling and absorbance was recorded at 700 nm when 0.5 ml of ferric chloride was added. Ascorbic acid was used as a standard compound.

2.4. *In-vivo* studies of *N. abortristis* flower extract

2.4.1. Experimental animals

The animals for the present study procured after animal ethical clearance from the Institutional Animal Ethical Committee (IAEC), in Pinnacle Biomedical Research Institute, Bhopal (M. P.) India. The animal experiments were carried out as per Control and Supervision of Experiments on Animals (CPCSEA) norms. In present study healthy albino wistar rats, 8-10 weeks old with 150-200 gm body weight were used for anti-arthritic activity. The animals were acclimatized in cages under standard environmental conditions of light/dark cycles (12 hours/12 hours) and temperature (23±1°C). The animals had free access to water *ad libitum* and a standard pellet diet.

2.4.2. Acute Oral Toxicity

The acute toxicity study [40] was performed for *N. abortristis* flower extract according to OECD (423) guidelines. Wistar rats were selected randomly. The test groups include four treatment groups with dosages at 5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg body weight. Individual doses were calculated on the basis of body weight of the animal on the day of treatment. The animals were observed almost constantly for behavioural changes, mortality and appearance during firstly for first 4 hours, periodically during the 24 hours and then every day for a period of two weeks.

2.4.3. In-vivo Chronic Inflammatory Disorders

Complete Freund's adjuvant (CFA) induced Arthritis and Monoiodoacetate (MIA) induced Arthritis model were used to evaluate the effect of plant drug on chronic inflammatory disorders.

2.4.3.1. Complete Freund's adjuvant (CFA) induced Arthritis

Freund's adjuvant induced arthritis model [41-42] was used to assess the anti-arthritic activity of the *N. abortristis* flower extract in wistar rats. The extract which shows the maximum antioxidant results was selected further for *in-vivo* studies. Animals were randomly divided into four groups of six animals each (n=6)., Group I received Freund's adjuvant (0.01 ml) served as arthritic control, Group II animals received Indomethacin (10 mg.kg-1 p.o.) served as reference standard, Group III animals received 200mg/kg lower dose of crude extract and Group IV animals received 400 mg/kg higher dose of crude extract. The paw volume is an indicator of arthritic condition. To assess the anti-inflammatory and anti-arthritic activity of the extracts were given to the animal 30 minutes before the administration of Freund's adjuvant and continued till 28th day. Paw volume was measured on 0th, 7th, 14th, 21th and 28th day by using electronic digital callipers. After 28th days, blood samples were collected by puncturing the retro-orbital plexus into heparinised vials and analysed for total leukocyte counts (TLC), differential leukocyte counts (DLC).

3. RESULTS AND DISCUSSION

3.1. Qualitative and Quantitative phytochemical screening

Results of phytochemical screening of ethyl acetate and methanolic extract of *N. abortristis* flowers showed the maximum presence of alkaloid, flavonoid, terpenoid, phenols and carbohydrates while glycosides are present in methanolic extract.

3.1.1. Total phenolic and flavonoidal Content

The phenolic and flavonoid content present in methanolic and ethyl acetate flower extracts of *N. abortristis* were determined by linear regression equation (y=0.0663x-0.6409) r2 = 0.9924, with their respective standard gallic acid and rutin.

3.2. Antioxidant activity of *N. abortristis* flower extracts

3.2.1. DPPH and Super oxide dismutase assay

Antioxidant activity of petroleum ether extract, methanolic extract and ethyl acetate extract were determined by DPPH, Super oxide dismutase and reducing power assay. IC_{50} value of DPPH and Superoxide assay are shown in Table 3 Figs. 1-3.

3.2.2. Reducing power assay

Diagram shows the dose-response curves for the reducing powers of the *N. abortristis* extracts. It was found that the reducing power activity also increased with increasing the concentrations.

Table 1: Preliminary Phytochemical Screening of *N. abortristis* flowers extract

Phytoconstituents	Petroleum ether	Methanol	Ethyl acetate
Alkaloid	Present	Present	Present
Flavonoid	Absent	Present	Present
Terpinoid and steroids	Absent	Present	Present
Saponins	Absent	Absent	Absent
Tannin/Phenols	Absent	Present	Present
Carbohydrates	Absent	Present	Present
Reducing sugars	Absent	Absent	Absent
Glycosides	Absent	Present	Absent
Protein & amino acids	Absent	Absent	Absent

Table 2: Total flavonoid and total phenol content of flower extracts of *N. abortristis*

Extract	Total Phenol	GAE/g	Total Flavonoid	RE/g
Methanol	0.321±0.002	129mg	0.395±0.002	303.333mg
Ethyl acetate	0.195±0.002	66.167mg	0.199±0.003	106.67mg

Values were performed in triplicates and represented as mean±SD

Table 3: DPPH and super oxide dismutase free radical scavenging activity of flower extracts of *N. abortristis*

Extracts	IC_{50} value μg/ml	
	DPPH assay	SOD assay
Ascorbic acid (Standard)	25.07	36.27
Petroleum ether	182.74	227.24
Methanolic	95..30	127.50
Ethyl acetate	112.22	153.22

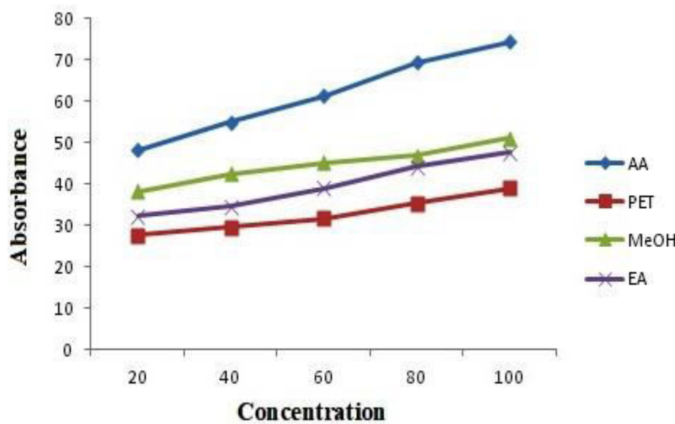


Fig. 1: Graphical representation of % inhibition of Ascorbic acid and *N.abortristis* extracts in DPPH assay.

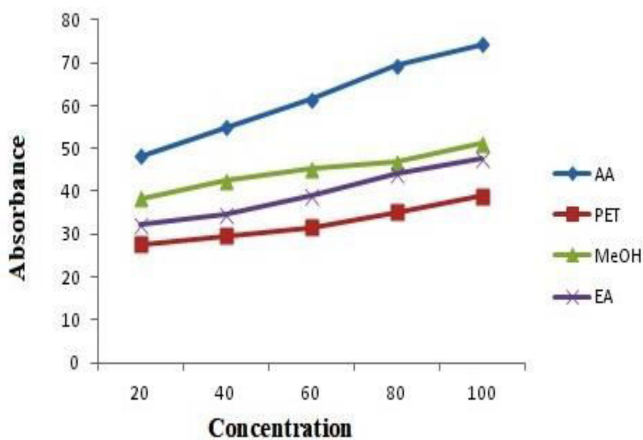


Fig. 2: Graphical representation of % inhibition of Ascorbic acid and *N. abortristis* extracts in superoxide assay

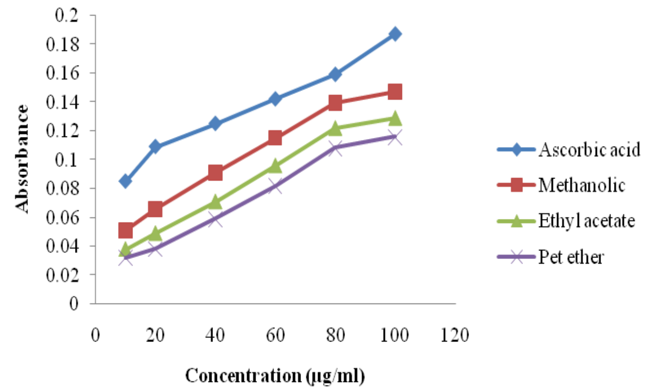


Fig. 3: Graphical representation of reducing power assay of *N. abortristis* flower extract

3.3. Acute Oral toxicity

Methanolic extract of *N. abortristis* flowers were employed as per OECD guideline in 3 single sex wistar rats. Extract did not show any mortality and toxicity at any concentration not even at highest does of 2000 mg. kg^{-1}. Therefore in present study, 200mg (low) and 400mg (high) doses of methanolic drug extract were selected for determination of anti-arthritic activity.

3.4. *In-vivo* anti-arthritis determination

Methanolic extract of *N. abortristis* extract shows the better antioxidant activity. So it was used in further *in vivo* study to determine the anti-arthritic of *N. abortristis* flower extract using Complete Freund's adjuvant (CFA) induced arthritis model.

3.4.1. Complete Freund's adjuvant (CFA) induced Arthritis

Effect of methanolic plant extract on Freund's adjuvant (CFA) induced arthritis was determined by measuring the paw volume for the duration of 0-28th days using electronic digital callipers and the estimation of total leukocyte and differential leukocyte count in blood sample.

3.4.1.1. Paw volume determination

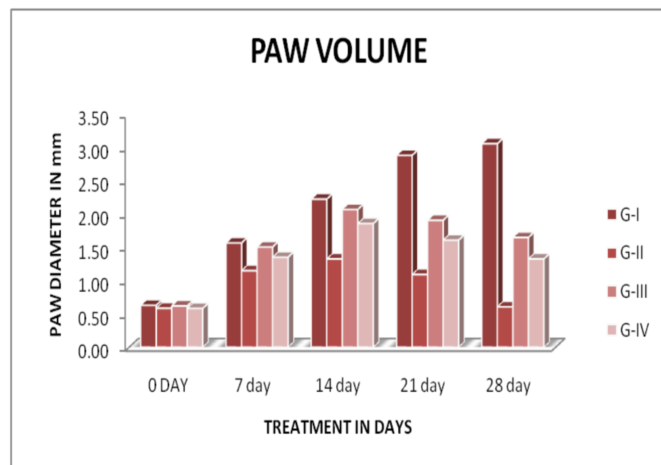
Oral administration of *N. abortristis* methanolic extract in 200mg/kg and 400mg/kg showed the manifesting effect on Freund's adjuvant induced chronic arthritic

rats. The noticeable effect was observed on 28th day at both lower and higher extract concentration and the mean±SD was 1.65±0.026 and 1.33±0.034, respectively while the standard drug Indomethacin shows the 0.61±0.04. The results are shown in table 5 with graphical representations in Fig. 4.

Table 4: Measurement of paw volume in Freund's adjuvant (CFA) induced arthritis animal model

Day	Group-I	Group-II	Group-III	Group-IV
00	1.90±0.022	0.82±0.028	1.47±0.045	1.27±0.025
07	3.15±0.043	1.51±0.038	2.41±0.031	2.05±0.018
14	3.97±0.035	1.68±0.019	2.99±0.023	2.55±0.008
21	4.49±0.047	1.26±0.014	2.66±0.024	2.24±0.022
28	4.92±0.043	0.93±0.018	2.20±0.018	1.76±0.014

Values were performed in triplicates and represented as mean±SD



Freund's adjuvant induced arthritic model was used in different group of rats. The Indomethacine (10 mg.kg-1 body weight) was used as a standard drug. The control animal was induced with saline (5 ml.kg-1 body weight). The anti-arthritic effect was tested in different time interval such as 14,16,18,20 and 22days.

Fig. 4: The anti-arthritic effect of methanolic extract of N. abortristis flowers (200 and 400 mg/kg body weight)

3.4.1.2. Hematological Test

At the last day of the treatment, the blood sample was collected from puncturing the retro-orbital plexus into heparinised capillary vials and analyzed for total leukocyte counts (TLC), differential leukocytes counts (DLC).

Table 5: Effect of *N.abortristis* flower extract on Haematological parameters

Groups	Total Leukocytes Count	Differential Leukocyte Count
Group I	12268.33±37.071	4369.33±34.558
Group II	5780.83±33.670	2227.33±32.020
Group III	9089.83±40.583	3778.00±29.672
Group IV	7904.67±31.747	3242.83±26.977

Each value represents mean ±SD

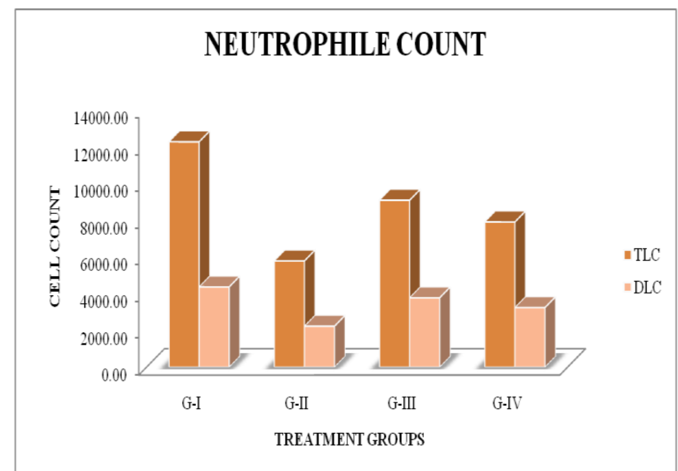


Fig. 5: Determination of the effect of N. abortristis flower extract on Haematological parameters (Total leukocyte count and Differential leukocyte count).

4. DISCUSSION

In present study, phytochemical estimation of *N. abortristis* flower samples were carried out in petroleum ether, ethyl acetate and methanolic solvents. Preliminary screening of all three extracts revealed the presence of phytochemicals which may have been responsible for biological activity. Phytochemicals plays an important role with easy availability, low toxicity, low cost, and their biological properties such as antioxidant activities, antimicrobial effects, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and antineoplastic properties [43]. In this study, methanolic extracts shows the presence of maximum active components, sequentially the ethyl acetate and petroleum ether extract (table 1). Total phenol and total flavonoid content was determined using standard procedures. Gallic acid and

rutin were used as standard compounds for phenol and flavonoid detection. Calculated Phenol content for ethyl acetate and methanol extracts was 66.167 and 129.0 mg GAE/g of dry extracts, respectively. Total flavonoid content expressed in terms of rutin equivalent for methanolic and ethyl acetate extract was 303.333 mg RE/g and 106.67 mg RE/g, respectively (table 2). Observed results of phytochemical constituents supported further the *in vitro* antioxidant and *in vivo* anti-inflammatory activity of *N. abortritis* flower extracts. There are different methods proposed to determine the antioxidant activity of plant chemicals such as metal chelation, free radical scavenging effects (DPPH), total antioxidant activity and reducing power assay. Super oxide anion radical, hydroxyl radical and hydrogen peroxide are widely used for activity of oxygen species destruction. *In vitro* antioxidant activity was determined using DPPH assay, superoxide dismutase assay and reducing power assay of crude extracts. The calculated IC_{50} value of ascorbic acid for DPPH and super oxide assay is 25.07 and 36.27 µg/ml, respectively. Petroleum ether extract of *N. abortristis* showed the higher IC_{50} value in both assay *i.e.*, 182.74 µg/ml and 227.24 µg/ml sequentially, the ethyl acetate and methanolic extract showed the 112.22 and 95.30 µg/ml in DPPH free radical scavenging activity while 153.22 µg/ml and 127.50 µg/ml for the super oxide scavenging activity. Figs. 1 & 2 show the graphical presentation of IC_{50} value for DPPH and superoxide dismutase assay. It was found that the reducing power activity also increased with increasing the concentrations. Methanolic extract shows the maximum reducing power as compared to ethyl acetate and petroleum ether extracts (Fig. 3). Hence, the methanolic extract showed the better results of antioxidant activity in comparison to petroleum ether and ethyl acetate flower extracts so it was used to determine the *invivo* anti-inflammatory activity of *N. abortristis* flowers. Dose dependent activity of extract was performed on the basis of OECD guidelines using single sex wistar rats. For the detection of anti-arthritic activity *in vivo* 200mg/kg lower and 400mg/kg higher dose was used on complete freund's adjutant induced arthritis animal model. To estimate the degree of inflammation and assessment of drug effect, paw volume measurement was apparently simple and quick procedure [45]. The perceptible result was observed in paw volume determination activity on 28th day at both lower and higher extract concentration *i.e.,* 1.65±0.026 and 1.33±0.034 respectively where as standard drug Indomethacin shows the 0.61±0.04. The results are shown in Figs. 3 and 4. Haematological parameter was also determined by total leukocyte count and differential leukocyte count after treating with methanolic extract of *N. abortristis* flowers. Extract in concentration of 400 mg/kg and 200 mg/kg showed significant results compared to standard drug Indomethacin.

On the basis of present study and obtained results it could be concluded that *N. abotristis* methanolic extract possess the efficient anti-oxidant and anti-inflammatory effect.

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