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ASSESSMENT OF LEAF EXTRACTS OF *ARTEMISIA NILAGIRICA* AGAINST ANTIOXIDANT, CYTOTOXIC AND PHYTOCHEMICAL ASSAYS.

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ABSTRACT

Asteraceae is one of the families of medicinal plants with large no of genus involved in traditional medicine. *A. nilagirica* is traditionally used in management of epilepsy, nervous disorder, as diuretic, anti–inflammatory and skin disorders. In the present study solvent leaf extracts of *Artemisia nilagirica* were explored for its Phytochemical, Antioxidant and Cytotoxic potential. Phytochemical test revealed the main constituents as Flavonoids, Saponin and Tannins. Cytotoxic activity was found highest in chloroform extract (52.40%) at the dose 100 µg/ml followed by acetone extract which showed 41.26% activity at the higher dose. In qualitative TLC based antioxidant assay, maximum no of antioxidant bands were obtained in Chloroform extract in the solvent system (Benzene: Ethanol: Amonium hydroxide(BEA).

KEYWORDS: Phytochemical, Antioxidant, Cytotoxic, TLC, *Artemisia nilagirica.*

INTRODUCTION

Artemisia nilagirica is commonly known as Indian Wormwood, sagewood and davana in Hindi [1]. Infusions of the leaves are applied as haemostatic and to allay the burning sensation in conjunctivitis. The roots are used as tonic and antiseptic.[2] The leaf extract of *A.nilagirica* showed antibacterial activity against the Gram negative bacteria. Ethanolic extract of flowering meristems of *A. nilagirica* was reported to be effective against the root knot disease of mulberry caused by *Melodigyne incognita*.[3] Traditionally it is used in the treatment of epilepsy, nervous disorders, as diuretic, as anti-inflammatory and various skin diseases.[4] Although plant has established medicinal potential as can be seen from the vast literature available on the plant. Plant was obtained from the southern state of India (Karnataka) and established in the medicinal germplasm garden of RPRC, Odisha so in the present study leaf extracts of the plant were explored for their phytochemical, antioxidant and cytotoxic activity so as to find out that change of habitat has any effect on the medicinal potential of plant or not.

MATERIALS AND METHODS

Collection and processing of plant materials

Fresh leaves of *Artemisia nilagirica* were collected from the medicinal germplasm garden of Regional Plant Resource Center (RPRC), Bhubaneswar. Leaves were washed with running tap water to remove dust and impurities followed by drying in shade. After complete drying of leaves they were ground in to fine powder by using mechanical grinder. Dried powder was further used for solvent extraction.

Moisture content determination

For the calculation of moisture content, Fresh weight of leaves was taken after collection and further weight was taken after they were completely dry. Moisture content of the leaves was calculated by using the following formula:

$$\textbf{Moisture content (\%)} = (F_w - D_w / F_w) \times 100$$

Where, F_w = Weight of the fresh plant sample

D_w = Weight of the dried powdered sample

Solvent extraction

Solvent extraction was conducted by the process of maceration. In maceration process 100 gm of leaf powder of *Artemisia nilagirica* was taken in a beaker and 500 ml of hexane solvent is added to it, mixture was stirred with a glass rod at regular intervals and left overnight for percolation. Extract was filtered using Whatmann filter paper. Same process was repeated for each solvent thrice. Collected extract was concentrated under vacuum in rotavapor(Buchii make) at lower temperature of 40-45℃. Concentrated extracts were stored in screw cap vials till further use.

Percentage of yield

Same was calculated for each of the extract as follows –

Percentage yield = (Extract weight / Powdered weight) x 100

Phytochemical analysis
Phytochemical tests were done by standard method[5] with both fresh leaves and with solvent leaf extracts.

Test for Alkaloids: Alkaloid tests were done by using 3 different reagents.

Dragendroff's test- To 1ml of leaf extract 2 ml of 1% HCl was added and then boiled for few minutes, after boiling 2-3 drops of dragendroff's reagent was added and sample was observed for redish brown precipitate.

Wagner's test- To 1 ml of leaf extract 1 ml of 1% H_2SO_4 was added followed by few drops of wagner's reagent. Formation of precipitate depicts the presence of alkaloids.

Mayer's test- To 1 ml of leaf extract 2 ml of 1% HCl and mayer's reagent was added drop wise and was observed for the formation of precipitate.

Test for flavonoid: To 2.5 ml of leaf extract 1 ml of 10% NaOH was added.From the side of the test tube, drops of conc. HCl were added. Yellow colour turns to colourless which indicates presence of flavonoids.

Test for Anthraquinone: To 1ml of leaf extract 2 ml of 5% KOH was added and was observed for pink colouration.

Test for Saponin: To 1ml of leaf extract 2 ml of $NaHCO_3$ was added and on shaking forms lather.

Test for Terpenoids: To 1 ml of extract 400µl of chloroform and 4-5 drops of conc.H_2SO_4 was added from the walls of the test tube. Redish brown ring depicts the presence of terpenoids

Test for Cardiac glycoside: To 2.5 ml of extract 2 ml of glacial acetic acid, few drops of $FeCl_3$ and conc.H_2SO_4 was added from the walls of the test tube. Presence of cardiac glycoside was determined by reddish brown ring.

Test for Tannin: It was conducted by two methods.
Method A – 1 ml of extract was boiled and few drops of $FeCl_3$ were added to it. The sample was observed for blue, black, green precipitate.

Method B – To 1 ml of extract 500µl of lead acetate was added which gives yellow colour in the presence of tannins.

Bioevaluation of solvent extracts
Antioxidant, cytotoxic and antifungal activities were tested for all the leaf extracts of *Artemisia nilagirica*.

Antioxidant Actvity
Qualitative Analysis (TLC based antioxidant studies)
To detect antioxidant activity qualitatively, 2,2-Diphenyl 1-picrylhydrazyl (DPPH) assay was carried out. TLC sheet (Silica gel 60 F254, Merck company) coated with silica gel was used as stationary phase.

For about 5 µl of each sample was loaded on TLC activated sheet, following solvent systems/mobile phases were used for TLC based antioxidant activity
a) Ethyl acetate : methanol : water (40 : 4.5 : 4) [EMW] (polar neutral)
b) Chloroform : ethyl acetate : formic acid (5 : 4 : 1) [CEF] (intermediate polarity/ acidic)
c) Benzene : ethanol : ammonium hydroxide (90 :10 :1) [BEA] (Non polar/basic)

It was sprayed with 0.2% DPPH in methanol as an indicator following standard protocol.[6]

The presence of antioxidant compounds were detected by yellow spots against a purple background on the TLC sheet sprayed with 0.2% DPPH in methanol.

DPPH + AH $\rightarrow$ **DPPH – H +A^-**
(Purple colour) **(Yellow colour)**

DPPH radical scavenging assay
The radical scavenging activity of different extracts against DPPH was determined spectrophotometrically by the standard method.[7] DPPH reacts with an antioxidant compound that can donate hydrogen and it gets reduced. The change in colour (from deep violet to light yellow) was measured. DPPH is a stable free radical and accepts an electron, or hydrogen radical to become a stable diamagnetic molecule. The intensity of the yellow colour depends on the amount and nature of radical scavenger present. A reaction mixture containing 500µl of 1 mM DPPH, various concentrations of plant extracts (7.81, 15.62, 31.25, 62.5, 125, 250 and 500 µg/ml) were prepared in methanol. A test tube containing only methanol and 500 µl of DPPH solution was taken as control. After 30 minutes incubation in dark, yellow colour chromophore was measured at 517nm. Ascorbic acid was used as standard. The percentage scavenging of DPPH free radical was calculated by following formula.
% scavenged DPPH radical = A control – A sample/A control X 100; Where A control is absorbance of control and A sample is absorbance of sample.

Cytotoxic activity (Brine shrimp lethality test)
Cytotoxic activity study was carried out by brine shrimp lethality assay.[8] Brine shrimp eggs were incubated in saline prepared by dissolving 3.6 gm of black salt in 200 ml distilled water. Stock solution of different extract were prepared at a concentration of 10 mg/ml, cytotoxic assay was carried out at three doses 50, 100 &200 µg/ml. For each dose level 3 replicates were used. Motility, readings were taken every hour up to 4 hrs. Motility was graded as below:
4+ = high motility, 3+ = motile, 2+ = sluggish, 1+ = slow and Nil = no activity at all

After 24 hrs. live larvae in the control and experimental tubes were counted and percentage inhibition was calculated using the following formula.

Percentage inhibition = No of Live larvae in experimental X 100
No of live larvae in controls

RESULTS AND DISCUSSION

The leaves of the plant were collected and the moisture content was estimated. It was found that moisture content of the plant leaves was 81.42%. Yield of the methanol extract was highest amongst the solvent extracts followed by hexane, chloroform and acetone (Table 1). Thus polar extracts were rich in comparison to non-polar extracts.

Table 1: Percentage of Yield.

Solvent Extracts	Percentage of Yield
HEXANE	1.85%
CHLOROFORM	1.46%
ACETONE	1.97%
METHANOL	5.94%

Phytochemical Screening

The phytochemical tests were carried out to test the presence of secondary metabolites in the different extracts (hexane, chloroform, acetone, and methanol). Out of seven phytochemical screened, three were found present in various solvent extracts. They were flavonoids, tannin, saponins (Table 2). Alkaloids, anthraquinones, cardiac glycosides and terpenoid were absent in the entire sample. Acetone extract and methanol extract showed three numbers of secondary metabolites followed by chloroform and hexane extract showed only one secondary metabolites. Fresh sample showed only tannins. Presence of flavonoids indicates antioxidant potential of the extracts as there is a direct correlation between the two.[8] Tannin have been reported for their antimicrobial activities against bacteria and fungi, and also have effect on oxygen radical scavenging[9], hence presence of same also indicates towards the medicinal potential of extracts.

Table 2: Phytochemical analysis of leaf solvent extracts of *Artemisia nilagirica.*

Phytochemicals	Fresh sample	Hexane	Chloroform	Acetone	Methanol
Alkaloids	_	_	_	_	_
Flavonoid	_	+	_	+	+
Anthraquinone	_	_	_	_	_
Saponin	_	_	_	+	+
Terpenoids	_	_	_	_	_
Cardiac glycosides	_	_	_	_	_
Tannin	+	_	+	+	+

+ indicates presence of phytochemical, - indicates absence of phytochemical.

Bioevaluation of extracts of Artemisia nilagirica

For biological evaluation two parameters were selected these were cytotoxic activity using brine shrimp assay and antioxidant activity using qualitative and quantitative antioxidant assays.

Cytotoxic activity using brine shrimp assay

All the extracts were tested in three doses (25, 50, 100 µg/ml). Cytotoxic activity was found highest in chloroform extract (52.40%) at the dose 100 µg/ml followed by acetone extract which showed 41.26% activity at the dose 100 µg/ml. Remaining extracts showed only mild activities against brine shrimp mortality assay (Fig 1). However all the extracts showed dose dependent activity i.e, activity increased with increasing the dose level. ED_{50} of chloroform extract was 72.5 microgram/ml. Thus, chloroform extract of the plant warrants further exploration.

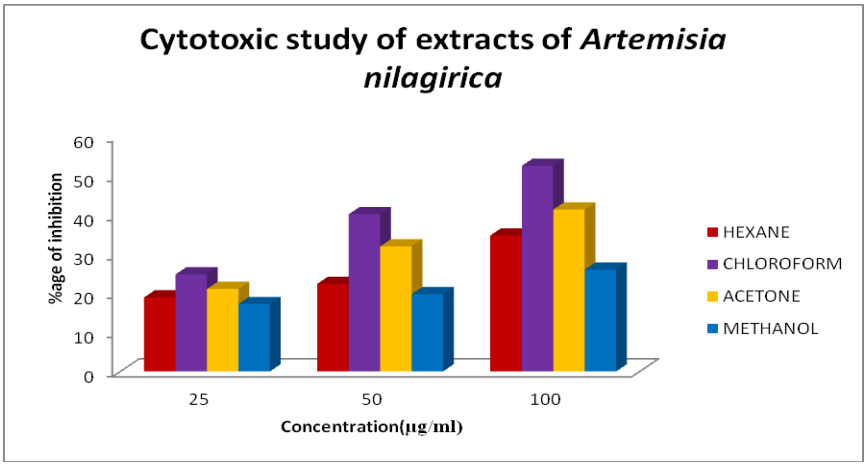

Fig 1: Cytotoxic activity of solvent extracts of *Artemisia nilagirica.*

Antioxidant activity of extracts of Artemisia nilagirica

TLC based qualitative antioxidant assay

The presence of antioxidant compounds were detected by yellow spot against a purple background on the TLC sheet sprayed with 0.2% DPPH in methanol. Number of yellow bands correspond to the number of antioxidant molecules in the sample. On the basis of TLC, maximum number of antioxidant bands were obtained in chloroform extract in BEA(11) solvent followed by acetone extract(7) in the same solvent(Table 3).

Table 3: Qualitative antioxidant assay of solvent extracts of *Artemisia nilagirica.*

SAMPLES	SOLVENTS	NO.OF BANDS	Rf VALUES
Ascorbic acid	BEA	1	_
	CEF	1	_
	EMW	1	_
Hexane	BEA	3	0.12,0.17,0.24
	CEF	0	_
	EMW	0	_
Chloroform	BEA	11	0.05,0.08,0.12,0.17,0.23,0.25,0.32,0.46, 0.67,0.92,0.97
	CEF	4	0.05,0.15,0.76,0.62
	EMW	3	0.25,0.37,0.49
Acetone	BEA	7	0.08,0.12,0.17,0.21,0.25,0.43,0.94
	CEF	5	0.08,0.17,0.26,0.78,0.92
	EMW	4	0.07,0.21,0.36,0.51
Methanol	BEA	3	0.11,0.15,0.20
	CEF	2	0.11,0.29
	EMW	5	0.20,0.32,0.43,0.48,0.53

Quantitative antioxidant activity

DPPH radical scavenging assay

As can be observed from Fig 2, None of the extract showed better antioxidant activity in comparison to the standard Ascorbic acid. However at the highest dose acetone and methanol extracts showed more than 80% antioxidant activity, which is quite good as extracts are mixture of a number of molecules, so it is possible that some antioxidant molecule is definitely there in these extracts and hence study has provided lead in the form of these two extracts for isolation of antioxidant principle.

Although maximum number of antioxidant bands were obtained in chloroform extract but overall antioxidant activity of the extract was not as promising, which clearly indicates that molecules act in antagonistic way to one another. Whereas in case of acetone and methanol extracts molecules showed good activity at higher dose suggesting the synergistic role of antioxidant molecules.

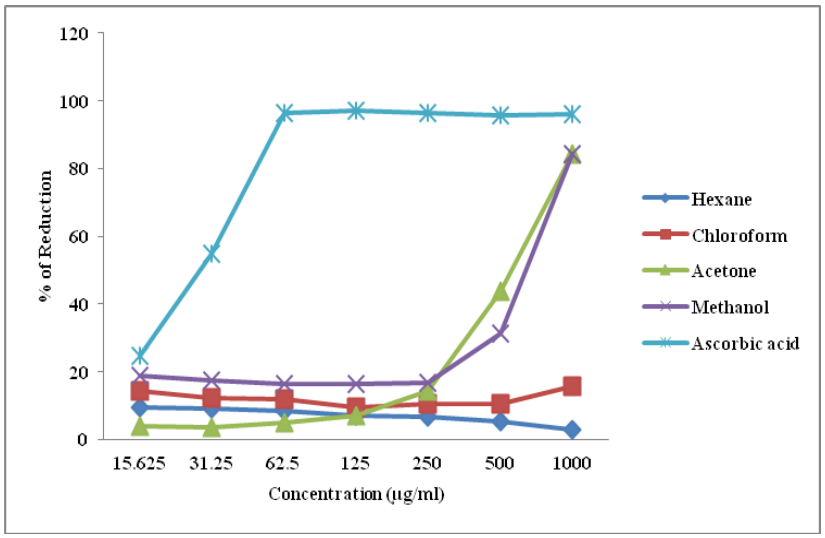

Fig. 2: DPPH free radical scavenging activity of leaf extracts of *Artemisia nilagiric.*

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