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Phytochemical screening and antibacterial activity of ethanolic extract of *Artemisia Nilagirica*

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Abstract

Artemisia Nilagirica (Clarke) plant posses various pharmacological properties like antileishmanial activity, antimalerial, anthelmintic, antiseptic, expectorant, astringent, and anti inflammatory. Phytochemical screening of *Artemisia Nilagirica* was carried out. Result indicate that the plant contain flavonoids, steroids, terpenoids, saponins, tannins, proteins, essential oil. Antibacterial evaluation of the plant was carried out and it found to have a good antibacterial action.

Key words: *Artemisia Nilagirica,* Antileishmanial, Antiinflammatory, Antimalarial, Anthelmintic

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Introduction

*Artemisia Nilagirica (Clarke) (*Hindi : Nagdona, Dauna, Tamil: Makkipu, Masipattiri, English: Indian Wormwood.) belongs to Asteraceae. It is the aromatic shrub found throughout the mountains districts of India. It grows at Mount Abu in Mar war and on the Western Ghats and some parts of South India. [1] A tall aromatic perennial shrurb, often gregarious, pubescent or villous throughout; lower leaves ovate in out line deeply pinnatisect with small stipule-like lobes at the base, pubescent above, white tomentose beneath, upper most smaller, 3-fid or entire, lanceolate; panicled racemer, outer flowers female, very slender, inner disk flowers fertile, bisexual, bracts ovate or oblong, margins scarious fruits oblong ellipsoid minute achene's.[2] Plant Contain sesquiterpene lactones, exiguaflavone A and B, macckianin and 2-(2,4-dihydroxyphenyl)-5,6-methylenedioxy benzofuran.[3] The crude methanolic and ethanolic extract of plant A. *Nilagirica (Clarke)* wile shows reasonably high potency against plasmodium falciparum.[4] It is also said to be anthelmintic, antiseptic and expectorant, astringent, aromatic, anti inflammatory, appetizer, digestive and diuretic. It is also used in cough, asthma, leprosy skin disease and as antiseptic. [5-7]

Materials and Methods

Plant *Artemisia Nilagirica* (Clarke) pamp is an herb commonly found in hilly districts. It was procured from Ooty and identified by the survey of medicinal plant and collection unit.

Extraction

The leaves and flowering top of *Artemisia nilagirica (Clarke)* was dried under shade and then crushed into powder with a mechanical pulveriser and was extracted with 95% ethanol in soxhlet apparatus about 55 hours. The solvent was removed by vacuum distillation under reduced pressure.

Phytochemical Screening

The ethanol extract of *Artemisia nilagirica (Clarke)* subjected to various color reaction to identify the nature of the components. [8-12]

Test for alkaloids

The small portion of ethanol extracts were stored separately with a few drops of dilute hydrochloric acid and filtered. The filtrate was tested with various alkaloidal agents, such as Mayer's reagent (cream precipitate) Dragendorffs reagent (orange brown precipitate).

Test for carbohydrates and glycosides

Small quantities of ethanolic extract were dissolved separately in 5 ml of distilled water and filtered. The filtrate may be subjected to Molisch's test to defect the absence of carbohydrates. Another small portion of extract was hydrolyzed with dilute hydrochloric acid for few hours in water-bath and was subjected to Liebermann- Burchard's, legal and Borntrager's test to defect absence of different glycosides.

Test for flavonoids

5 ml of dilute ammonia solution were added to a portion of aqueous filtrate of plant extract followed by addition of concentrated H_2SO_4. A yellow coloration absorbed in extract indicated presence of flavonoids. The yellow coloration disappeared on standing.

Test for steroids

(2ml) Two ml of acetic anhydride was added to 0.5 g ethanolic extract with 2ml H_2SO_4. The color changed from violet to blue or green in samples indicated presence of steroid.

Test for terpenoids (salkowski test)

Five ml of extract was mixed in 2 ml of chloroform, and concentrated H_2SO_4 (3ml), was carefully added to form a layer. A reddish brown coloration of the interface was formed indicated presence of terpenoids.

Test for saponin

About 1 ml of alcoholic and agrees extract was diluted with distilled water to 20ml and shaken in graduated cylinder for 15 minutes. One cm layer of foam indicated presence of saponin.

Test for tannin Vanillin-hydrochloric acid test (Vanillin 1 g, alcohol 10 ml, concentrated hydrochloric acid 10 ml). When a drug is treated with vanillin-hydrochloric acid reagent, pink or red color is formed due to formation of phloroglucinol.

Test for protein

Millon's reaction: Million's reagent (mercuric nitrate in nitric acid containing a trace of nitrous acid) usually yields a white precipitate on addition to a protein solution which turns red on heating. This reaction is characteristic of phenols (e.g. the phenolic amino acid tyrosine).

Test for volatile oil

Place a thick section of drug on glass slide. Add a drop of Sudan red 3rd reagent and after two minute wash with 50% alcohol mount in glycerin. In microscope, oil globules appear red color.

Antibacterial Activity

In the present research work, the antibacterial activity spectrum of ethanolic extract of *Artemisia Nilagirica* was analyzed.[13-16] Three Gram positive bacteria, *Staphylococcus aureus* (MTCC 737)**,** *Enterococcus faecalis* (MTCC 439), *Bacillus cereus* (MTCC 430) and three Gram negative bacteria *Klebsiella pneumoniae* (MTCC 109), *Pseudomonas aeruginosa* (MTCC 2642), *Escherichia coli* (MTCC 1687) were used. Inoculum size was adjusted to 1 to 2 × 10^7 CFU (Colony Forming Units)/ml by serial dilution with sterilized nutrient broth media. Nutrient agar (pH 7.2-7.4) was used for routine susceptibility testing of nonfastidious bacteria. Stock solution of 10000µg/ml was prepared in 20 % v/v water in DMSO. Using the stock solution, 6000µg/ml, 4000µg/ml, 2000µg/ml and 1500µg/ml solutions were prepared from which 100 µl solution was taken for assay. Ciprofloxacin was used as a standard. 20 % v/v WFI in DMSO was used as a control. Antibacterial assay was carried out by agar Well Diffusion Method. [17-19] After 16 to 18 hours of incubation, each plate is examined.

Results and Discussion

Phytochemical Study

The phytochemical studies revealed the presence of flavonoids, steroids, terpenoid, saponins, tannins, proteins and essential oil.

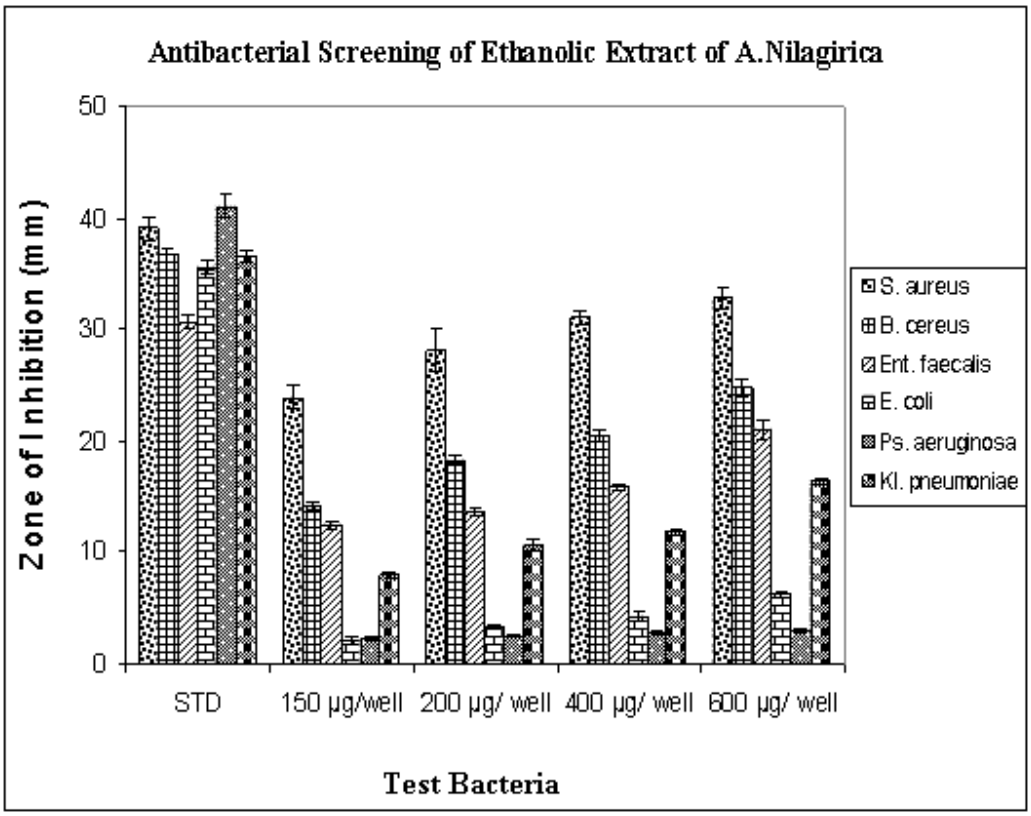
Table 1. Phytochemical Composition observations of ethanolic extract of *Artemisia nilagirica (Clarke) extract*

Phytochemicals	Ethanolic Extract
Alkaloid	-
Carbohydrate	-
Glycoside	-
Flavonoid	+
Steroid	+
Terpenoid	+
Saponins	+
Tannins	+
Protein	+
Essential oil	+

Table 2. Zone of inhibition of ethanolic extract of *Artemisia Nilagirica*.

	S. aureus	***B. cereus***	***Ent. faecalis***	***E. coli***	***Ps. aeruginosa***	***Kl. pneumoniae***
STD	39.10 ±0.95	36.67 ± 0.61	30.67± 0.61	35.60 ± 0.53	41.07± 1.01	36.53 ± 0.61
150 µg/well	23.93 ± 1.03	14.13 ± 0.41	12.47 ± 0.42	2.00 ± 0.40	2.20 ± 0.20	7.93 ± 0.31
200 µg/ well	28.23± 1.86	18.33 ± 0.31	13.60 ± 0.35	3.33 ± 0.15	2.47 ± 0.12	10.60 ± 0.60
400 µg/ well	31.07 ± 0.72	20.53 ± 0.61	15.80 ± 0.20	4.27 ± 0.31	2.80 ± 0.20	11.87 ± 0.31
600 µg/ well	32.90 ± 0.95	24.80 ± 0.80	21.00 ± 0.87	6.30 ± 0.26	3.00 ± 0.20	16.33 ± 0.31

Figure 1. Graphical presentation of zone of inhibition against test microorganism



The results of preliminary evaluation showed that *Artemisia Nilagirica* posses good antibacterial activity. *P. aeruginosa* and *E. coli* are resistant or less susceptible to *Artemisia Nilagirica*.

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