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PHYTOCHEMICAL SCREENING OF ETHANOLIC EXTRACT OF *ARTEMISIA NILAGIRICA*

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ABSTRACT

Artemisia Nilagirica (Clarke) plant posses various pharmacological properties like antileishmanial activity, antimalarial, anthelmintic, antiseptic, expectorant, astringent, and anti-inflammatory. Phytochemical screening of *Artemisia Nilagirica* was carried out. Results indicate that this plant contains flavonoids, steroids, terpenoids, saponins, tannins, proteins and essential oil. Column chromatographic study of ethanolic extract of *Artemisia Nilagirica* was performed and sesquiterpene lactones and flavonoid were isolated and characterized by IR, Mass, NMR spectroscopic data.

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

INTRODUCTION

Artemisia Nilagirica (Clarke) (Hindi:Nagdona, Dauna, Tamil: Makkipu, Masipattiri, English:Indian Warmwood.) belongs to Asteraceae. It is the aromatic shrub found throughout the hilly districts of India. It grows at Mount Abu in Marwar and on the Western ghats and some parts of South India[1]. A tall aromatic perennial shrub, often gregarious, pubescent or villous throughout; lower leaves ovate in outline 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 contains sesquiterpene lactones, exiguaflavone A and B, macckianin and 2- (2, 4- dihydroxyphenyl) - 5, 6 methylenedioxybenzofuran.[3]

The crude methanolic and ethanolic extracts of plant *A. Nilagirica* (Clarke) wile

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shows reasonably high potency against *plasmodium falciparum*. It is also said to be anthelmintic, antiseptic, expectorant, astringent, aromatic, antiinflammatory, appetizer, digestive and diuretic. It is also used in cough, asthma, leprosy, skin disease and as antiseptic[2].

EXPERIMENTAL

Materials and methods

Plant *Artemisia Nilagirica* (Clarke) pamp is a 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. It was crushed into powder with a mechanical pulveriser and then extracted with 95% ethanol in Soxhlet apparatus for about 55 hours. The solvent was removed by vacuum distillation under reduced pressure and resulting

Phytochemical screening

The ethanol extract of *Artemisia Nilagirica* (Clarke) was subjected to various color reaction to identify the nature of the components.[4-8]

Test for alkaloids

A small portion of ethanol extracts was 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) and 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 was 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 detect absence of different glycosides.

Test for flavonoids

5 mL of dilute ammonia solution was added to a portion of aqueous filtrate of plant

extract followed by addition of concentrated H_2SO_4. A yellow coloration in extract indicated presence of flavonoids. This yellow coloration disappeared on standing.

Test for steroids

Two mL of acetic anhydride was added to 0.5 g ethanolic extract with 2 mL H_2SO_4. The color changed from violet to blue or green in samples indicating the presence of steroid.

Test for terpenoids (Salkowski test)

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

Test for saponins

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

Test for tannins

Vanillin-hydrochloric acid test (Vanillin 1 g, alcohol 10 mL, concentrated hydrochloric acid 10 mL). When extract was treated with vanillin-hydrochloric acid reagent, a pink or red color is formed, which is due to formation of phloroglucinol.

Test for protein

Million'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

A thick section of drug on a glass slide was placed and a drop of Sudan red (3^{rd} reagent) was added. After two minutes, it was washed with 50% alcohol mounted in glycerin. In microscope, oil globule appear with red color.

Development of chromatogram

The ethanolic extract of *Artemisia Nilagirica* (Clarke) was subjected to column

chromatography over silica gel 60-120 mesh. The column was eluted with solvents of increasing polarity. The solvent used were petroleum ether (60-80^{o}C), benzene, chloroform and methanol. The fraction were collected in 10 mL each and allowed to evaporate to get the residue. Each fraction was tested for the presence of various constituents by thin layer chromatography for number and types of constituents with various solvent systems.

Isolation by column chromatography

By partitioning ethanol extract of *Artemisia Nilagirica* (Clarke) between benzene and ethyl acetate, isolated compound was named as AN-1. From TLC basis, column chromatography was carried out. By gradient elution technique in chloroform : methanol (60 : 40), the fraction No. 330-339 was collected and named as AN-2

RESULTS AND DISCUSSION

Phytochemical study

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

Table 1: Phytochemical composition of ethanolic extract of *Artemisia Nilagirica* (Clarke)

Phytochemicals	Ethanolic extract
Alkaloid	–
Carbohydrate	–
Glycoside	–
Flavonoid	+
Steroid	+
Terpenoid	+
Saponin	+
Tannin	+
Protein	+
Essential oil	+

Table 2: IR and UV spectroscopic data of the isolated samples

Sample	IR Spectra (cm^{-1},)	Wavelength (nm)	Absorbance	M.P (^oC)
AN-1	2956.67 (Ar. C-H), 1458.0 (C=C stretch) 1687.60 (C=O stretch), 1375.15 (Phenolic OH)	259	0.813	154-156
AN-2	3267.14 (Ar. C-H), 1513.37 (C=C stretch) 1633.59 (C=O stretch), 1384.79 (Ar. C-O-C-)	252	0.901	176-178

Mass and NMR spectral for AN-2

m/z: 279,149,167, 129, 111, 97 and 83

^{1}H NMR: Aromatic ring (δ ppm) 7.33 (7-CH), 6.90 (6-CH), 7.78 (5-CH), 6.85 (8-CH)

The mass spectrum has finally confirmed the structure by showing molecular ion peak of the compounds with fragmentation pattern. By NMR, the number of hydrogens of sample AN-2 was identified. Hence, it was concluded that the isolated compound AN-1 and AN-2 are sesquiterpene lactone and flavonoid, respectively.

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