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A REVIEW ON THE PHARMACOGNOSTICAL, ETHNOMEDICAL, PHYTOCHEMICAL AND PHARMACOLOGICAL ASPECTS OF *AYAPANA TRIPLINERVIS* (VAHL) R.M.KING & H.ROB.(ASTERACEAE)

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ABSTRACT

Ayapana triplinervis (Vahl) R. M. King & H. Rob also known as *Eupatorium triplinerve* is an ornamental perennial herb that belongs to the Asteraceae family. It is native to South America and can appear in the Amazon region of Brazil, Ecuador, Peru, and the three Guyanas. It is frequently known as white snake root, Aya-pana, water hemp, pool root etc. The flowers are pale pink and stem is reddish in colour which is hairless and thin. The plant parts often used are leaf and stem which has a vital importance in the folkloric treatment of piles, chronic diarrhoea, lung desease, influenza, constipation and badly infected wounds and bleedings. The plant is assumed to be sudorific, astringent, stomachic, stimulant, febrifuge, tonic and anti-tumorous. Phytochemical Analysis discloses the existence of alkaloids, phenols, Tannis, terpinoids and flavonoids. The plant shows pharmacological activities like anti-microbial, anti-viral, antinociceptic and inflammatory. The present review outlines all the research works that includes the pharmacognostic, phytochemical, ethno-medical and pharmacological aspects achieved on this plant for the sake of providing upgraded information for the time ahead.

KEYWORDS: *Ayapana triplinervis*, anti-microbial, *Eupatorium triplinerve*, *Eupatorium ayapana*.

INTRODUCTION

Medicinal plants play a crucial role in disease prevention and health care since ancient times. They are the major source of molecules with medicinal properties and has the residence of phytochemical constituents that promote healing process.[1] The utilization of plant based drugs are increasing around the world.[2] Root, stem, leaves, fruits, and flowers of various plants were found to possess secondary metabolites to show bioactivity.[3] The therapeutic potency of the plants, most particularly the anti-microbial activities are due to a wide verity of these secondary metabolites.[4] The most important secondary metabolites include terpenoids, phenolics, flavonoids, tannins, alkaloids and glycosides which act as an important source for single bioactive ingredients.[5] Phenolic compounds such as eugenol have been established for anti-fungal and anti-bacterial activities.[6] Over the past several years, tannins have been the focus of a lot of research since it can heal or prevent a number of ailments.[7] Quinine, an alkaloid, is popular for its antimalarial activity and many literatures indicates that plant alkaloids have considerable biological activity.[8] Flavonoids are used as anti-inflammatory, antispasmodic, anti-allergic and antimicrobial agents.[9] Likewise each secondary metabolites have a significant contribution in the traditional medicine. Synthetically prepared medicines act spontaneously but they produce unfavourable effects. Whereas medicinal plants work in a consolidated manner with no adverse effect on the body.[10] World health organisation reported that 80% of the world's population anticipate on traditional medicine for their primary health care needs which involves plant extract and their active components.[11]

Indian folklore medicine has a wide range of plants. *Ayapana triplinervis* (Vahl) R. M. King & H. Rob is one among them also known as *Eupatorium triplinerve* belongs to the family Asteraceae is an ornamental perennial herb. Most commonly known as Aya-pana, white snake root water hemp, pool root etc. It is native to south America and can appear in the Amazon region of Brazil, Ecuador, Peru, and the three Guyanas. It has a wide range of medicinal uses especially in folk medicine. The plant is traditionally used as antiseptic, antitussive, antiulcerous, astringent, cardiotonic, hemostat, hepatoprotector, laxative, sedative, stimulant, stomachic, tonic, sudorific. The phytochemical analysis of *Eupatorium triplinerve* was evaluated and reported thus used as an anti-bacterial and anti-ulcer agent.[12] Even if regional conventional healers have knowledge on this plant, scientific validation of the medicinal value has not been done considerably.

TAXONOMICAL CLASSIFICATION

Division: Phanerogamae

Class: Dicotyledonae

Sub class: Gamopetalae

Series: Inferae

Order: Asterales

Family: Asteraceae

Binomial: *Eupatorium triplinerve* vahl

PHARMACOGNOSTIC FEATURES

Botanical Description

Ayapana triplinervis is an ornamental perennial herb that grows upto 30 to 60 centimetres in height. This plant is half woody at the base, creeping and rooting at the lower parts. The stem is reddish brown in colour and hairless. Leaves are smooth, opposite, sub sessile, lanceolate, 3-nerved, acuminate, and glabrous inflorescence. Leaves are 5 to 8 cm long and is a small herb that has a strong smell. It consist of many flowering heads 6 to 13 milimeter long and contains about 20 flowers. They are mostly pale pink in colour. The fruits are achenes, narrowly oblong, 5-angled and about 2 milimeters length.

Microscopic characters

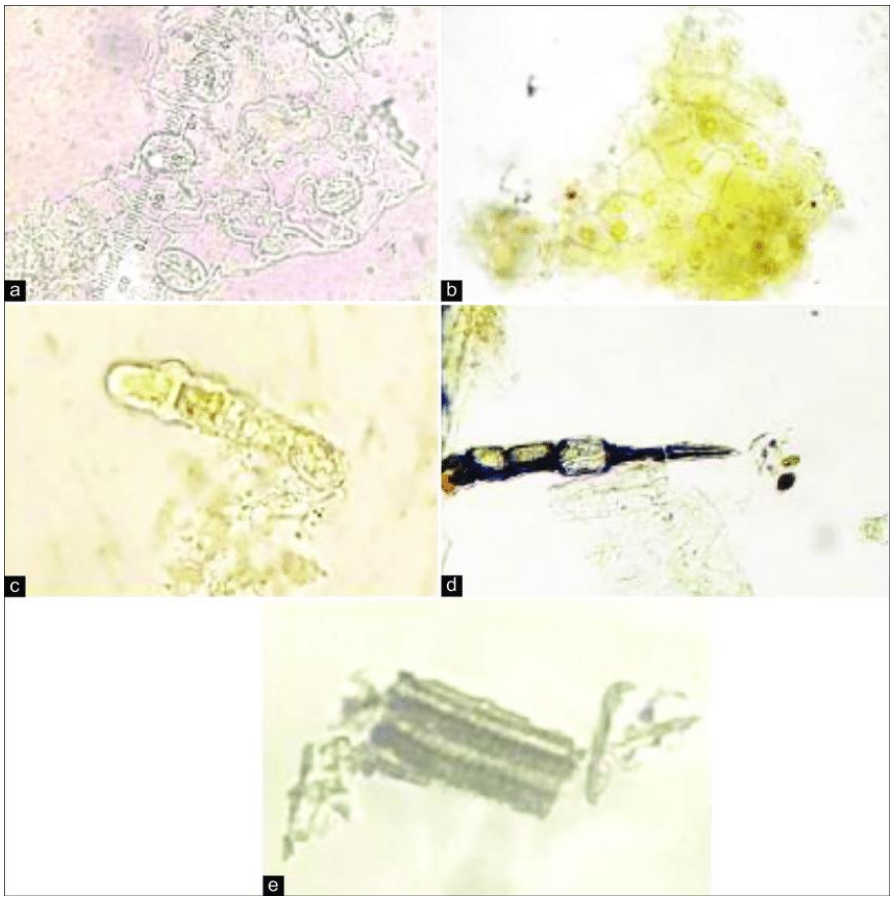

Figure 1: Microscopic examination of *Ayapana triplinervis* leaf powder (×100): Lower epidermal fragments with corrugated walls, anisocytic stomata, and Compositae glandular hair (a), upper epidermal fragments with polygonal walls, oil drops (b), glandular hair (c), multicellular hair (d), vessels with spiral thickening (e).[13]

Ethno-medical uses of *Ayapana triplinervis*

Ethnomedical studies reveal that plant and its decoction widely used for medicinal purpose in countries like Brazil, India, Sri Lanka, Bangladesh, Mauritius, West Indies, Peru, and Europe. The leaves and the whole plant were used as traditional medicine in India and several parts of the world. It is commonly used in Brazilian folk medicine as sedative, febrifuge, stimulant, anti-inflammatory and tonic.[12] It is also found in Puetro Rico and Hawaii and has been reported as a stimulant and tonic in small doses and a laxative when taken in quantity. A hot infusion has been found to be useful in the treatment of yellow fever in America and as a popular haemostatic remedy against various kinds of hemorrhage. The essential oil of this plant growing in Bangladesh found out to have moderate antimicrobial and strong antifungal activity.[14] In West Bengal the leaves of *Ayapana triplinervis* is used for dysentery and blood

enteritis. In Mauritius Islands the leaves of this plant is used for vomiting, diarrhea, stomach pain and colitis.[15] Ayapana is thought to be antineoplastic and used for cancerous tumors in both Peru and Argentina. An infusion of the entire plant is also used in Argentina to stimulate menstruation. The leaves of Ayapana are topically used for skin care by the Dayak Tribes, a native people of East Kalimantan.[16]

PHYTOCHEMICAL STUDIES

The qualitative phytochemical screening of the plant extracts shows the presence of various phytoconstituents that are responsible for the medicinal use of the plant. The acetone leaf extract of *Ayapana triplinervis* possess glycoside, terpenoids, phenols, flavonoids, reducing sugar, protiens, carbohydrates and alkaloids but the absence of steroids and saponins.[17] In the phytochemical screening using various extract of the fresh leaves, the petroleum ether fraction showed higher amounts of sterols, terpenes and free phenols, whereas the chloroform– methanol fractions had higher concentrations of alkaloids, tannins, phenols and flavonoids. The hexane fraction exhibited weak reactions for all the identification tests. While in methanolic fraction phenols and tannins were present in low amount.[18] In the hydroalcoholic extract of the whole plant, show the presence of saponin, reducing sugars, alkaloids, phenols, tannins, steroids, coumarin derivatives, and sesquiterpene lactones and the absence of polysaccharides and flavonoids. While in the phytochemical screening of different *E. triplinerve* leaf extracts of increasing polarity such as hexane, ethyl acetate and methanol, the methanol extract of the leaves presented better antimicrobial activity compared with other extracts.[19] The leaf powder extracted with different solvents like petroleum ether, benzene, chloroform, acetone, methanol and water, maximum phytoconstituents were showed in ethanol and water extract and anthroquinone glycosides, fats, fixed oils and phytosterols were absent. Extraction performed using n-hexane, ethyl acetate, and methanol as the solvents, methanol extracts had high antioxidant activity. Moreover, the methanol extract of the leaves had the highest levels of flavonoids and phenols and the secondary metabolites present in the methanol extract were alkaloids, flavonoids, terpenoids, tannins, and saponins.[20] In the case of methanolic extract of fresh leaves of *Ayapana triplinervis*, saponins, amino acids and reducing sugars are completely absent. Polyphenols and tannins are present in lesser amount. The ethanolic leaf and callus extract of *Eupatorium triplinerve* was shown more positive for the presence of natural chemical constituents followed by other solvents namely acetone, aqueous, petroleum ether and chloroform. Also it showed the presence of high tannin content in the callus extract.[21]

Chemical constitutions

Eupatorium triplinerve include 7-methoxy coumarin (herniarin), ayapin, carotene, vitamin-C and stigmasterol were isolated from its leaves. Other constituents include hydrangetin, daphnetin, daphnetin-7-methyl ether dimethyl ether, and umbelliferone. The other important compounds are thymoquinone and terponoids that are present in high percentage in this plant.[21]

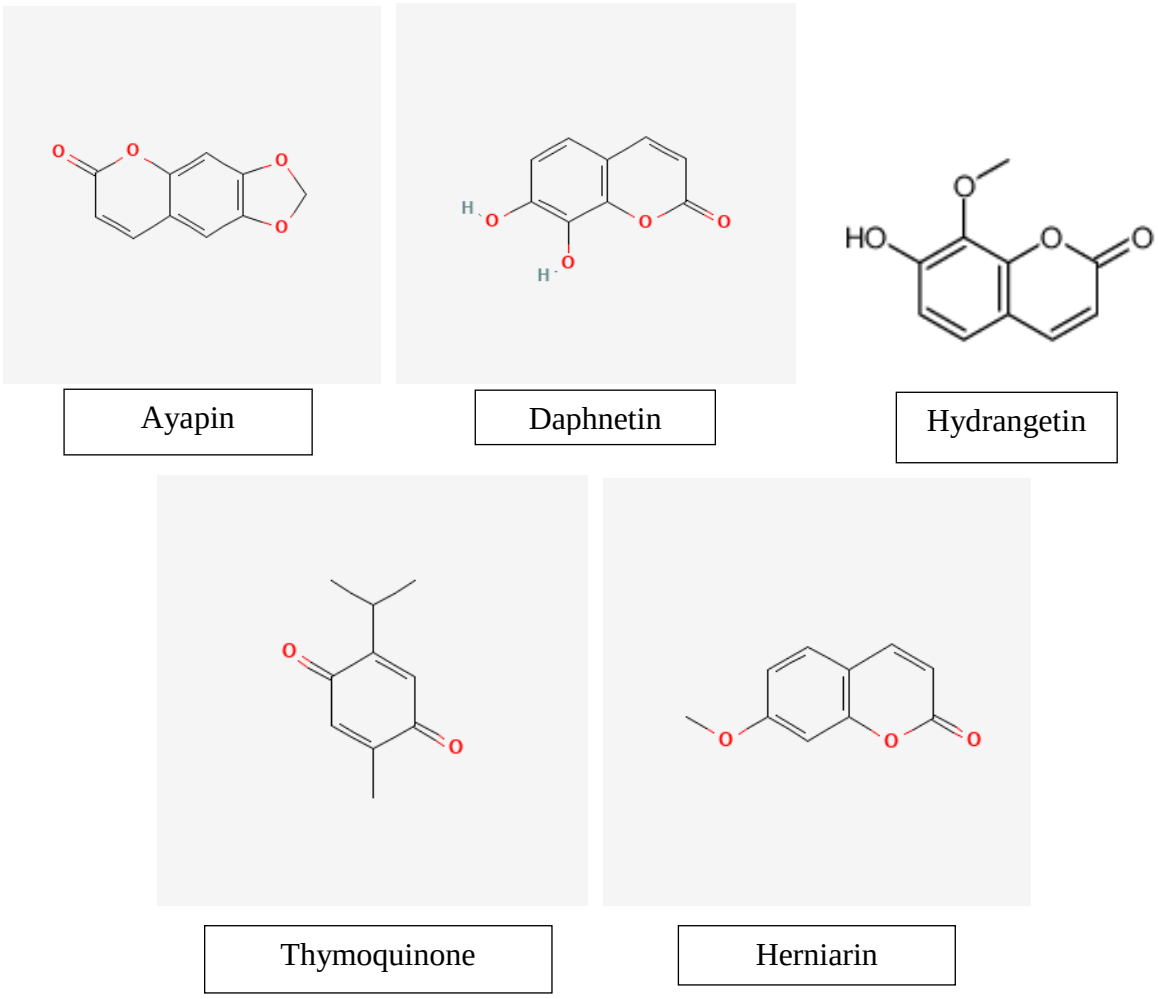

Figure 2: Structure of chemical constituents of *Ayapana triplinervis*.

Pharmacological Studies

Hepatoprotective activity

The hepatoprotective activity of methanol extract of *Eupatorium triplinerve* was evaluated in wistar albino rats. The extract produced significant ($p < 0.05$) hepatoprotective effect by decreasing the activity of serum enzymes, bilirubin, uric acid, and lipid peroxidation and significantly ($p < 0.05$) increased the levels of SOD, CAT, GSH and protein in a dose

dependent manner. Methanol extract of *Eupatorium triplinerve* possess potent hepatoprotective activity.[22]

Anthelmintic activity

The anthelmintic activities of *Eupatorium triplinerve* was evaluate on Indian Earthworm and round worm. *Eupatorium triplinerve* exhibited a dose dependent anthelmintic activity in both the models at the concentrations of 50mg/ml and 100mg/ml, thus indicating a broad spectrum of action.[23]

Haemostatic effect

Fresh juice and methanolic extract were evaluated for its haemostatic effect in rat model. Both fresh juice and methanolic extract decreased the bleeding time and clotting time significantly at dose of 200 mg/kg and 50 mg/kg, respectively. The use of ayapana in arresting blood bleeding traditionally was conformed.[24]

Evaluation of α-Glucosidase Inhibitory Activity

The antidiabetic compounds from the leaves of *Eupatorium triplinerve* were isolated from the methanol leaf extract. There were almost ten compounds such as β-sitosterol (1), stigmasterol (2), β-sitosterol 3-O-β-Dglucopyranoside (3), ayapanin (4), ayapin (5), thymoquinol 5-O-β-D-glucopyranoside (6), thyrsifloside (8), (E)-4-methoxymelilotoside (9), and kaempferol 3,7-di-O-β-D-glucopyranoside. In the *in vitro* test for α-glucosidase, inhibition of compounds 4–9 was seen and compounds 4, 5, and 7 showed the enzymatic inhibition respectively.[25]

Anticancer Activity

The anticancer activity of methanol leaves extract of *Eupatorium triplinerve* was evaluated against lung cancer cell line A-549 and normal cell line Vero. Screening for its cytotoxicity was conducted by MTT assay. The percentage of cell viability was reduced with the increased concentration, as evidenced by cell death. *Eupatorium triplinerve* extract shows potential cytotoxic activity with CTC50 of 450 µg/ml and 110 µg/ml against A-549 and Vero cell line. The crude extract showed moderate activity against cancer cells and it was less toxic to the normal cells.[26] Antitumour activity of the ethanolic and water extracts of the Eupatorium triplinerve was evaluated in Ehrlich Ascites Carcinoma (EAC) bearing Swiss albino mice. The activity was assessed by evaluating tumor volume, viable cell count, increase in body weight, mean survival time and hematological parameters of the EAC

bearing host. The both extracts at 150 mg/kg body weight have potent antitumour activity and ethanolic extract is comparatively more potent than the water extract.[27]

Antioxidant Activity

Antioxidant activity was done based on the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay using a UV–visible spectrophotometer and based on ferric reducing antioxidant power (FRAP) assay using a microplate reader. Different solvents such as n-hexane, ethyl acetate, and methanol were used. The DPPH assay using a UV–visible spectrophotometer, n-hexane, ethyl acetate, and methanol extracts with a final concentration of 25 µg/mL inhibited DPPH radical production by 38.91, 51.03, and 54.06%. FRAP assay using a microplate reader, the n-hexane, ethyl acetate, and methanol extracts had ferrous equivalent antioxidant capacity values of 460, 828.99, and 940.22µmol/g. The methanol extract shows the greatest antioxidant activity.[28] The Kupchan fractions of methanolic extract of *Eupatorium triplinerve* were investigated for its antioxidant activity. A partition of the crude extract was done by Kupchan protocol with methanol, petroleum ether, chloroform, carbon tetrachloride, methanol, and aqueous soluble materials. The Pet-ether soluble fraction demonstrated highest antioxidant activity (IC50 = 1.67 µg/ml).[29] The antioxidant activity of the plant was evaluated using extracts of hexane, CH2Cl2 and EtOAc as solvent. The EtOAc extract exhibited excellent activity against DPPH radicals with the half inhibition concentration (IC50) value at 22.7 µg/ml.[30]

Thrombolytic activity and Membrane stabilization activity

The Kupchan fractions of methanolic extract of *Eupatorium triplinerve* were evaluated for thrombolytic activity and membrane stabilization activity. A partition of the crude extract was done by Kupchan protocol with methanol, petroleum ether, chloroform, carbon tetrachloride, methanol, and aqueous soluble materials. Pet-ether and chloroform soluble partition revealed significant thrombolytic activity with 15.75% and 10.41% clot lyses. For membrane stabilizing activity, the carbon tetrachloride soluble fraction displayed the highest percentage inhibition of hemolysis.[29]

Antibiofilm and Anti-inflammatory activity

E. ayapana leaves were extracted with hexane, CH2Cl2, and EtOAc solvents to evaluate its antibiofilm and Anti-inflammatory activity. All the extracts exhibited pronounced anti-biofilm formation against *Escherichia coli*. The CH2Cl2 and EtOAc extracts exhibited

moderate anti-inflammatory activity against nitric oxide in RAW264.7 cells with IC50 values of 65.7 and 66.9 µg/ml.[30]

Anti-nociceptic Activity

The hydroalcoholic extracts of *Eupatorium triplinerve* was evaluated for its anti-noceiceptic activity using two-month-old male Wistar rats and Swiss male and female mice. Chemical (acetic acid and formalin) and thermal models of nociception were used. It shows antinociceptive effects was not related to opioid system.[31] The anti-nociceptive activity of 7-methoxy coumarin isolated from ethyl acetate fraction of the alcoholic extract of *Eupatorium triplinerve* was evaluated. A dose dependent anti-nociceptive action of 7- methoxy coumarin was revealed.[32]

Neurobehavioral effect

The putative effects of *Eupatorium triplinerve* on the central nervous system (CNS), including locomotor and anxiety activity, depression-like behaviour was evaluated in two-month-old male Wistar rats and Swiss male and female mice using hydroalcoholic extract. open-field (OF), elevated Plus-maze (EPM), and forced swimming tests were the behaviour essay used. *Eupatorium triplinerve* exerted mild sedative, anxiolytic and antidepressive effects on the CNS.[31]

Antibacterial Activity

Antimicrobial activity of *E. triplinerve* extracts from different parts of the plant were identified. The antimicrobial activity of the different extracts was performed by microdilution assay and the minimum inhibitory concentration (MIC) and minimum bactericidal concentration. The methanol extract of leaves presented the highest content of coumarins and lower MIC values of 62 and 75 mg/mL against Enterococcus faecalis and Staphylococcus aureus. its non-polar fractions showed antimicrobial activity with MIC ranging from 16 to 125 mg/mL against Gram-negative bacteria, mainly Escherichia coli. The non-polar fractions of *E. triplinerve* methanolic extract has better antimicrobial activity.[33] The antimicrobial activity of the essential oil was evaluated against ten bacteria (including Gram positive and Gram negative). The oil exhibited moderate antibacterial activities against all the test pathogens. The highest antibacterial activity was recorded against *Salmonella typhi* followed by *Shigella sonnei*.[14] Silver nanoparticles were produced using leaf extracts of *Ayapana triplinervis* to identify its antimicrobial activity. The antibacterial activity against common

pathogens was evaluated by well diffusion method. *Salmonella, Pseudomonas and Bacillus* gave positive results.[34]

Gastroprotective effect

The gastroprotective effect of *Ayapana triplinervis* leaves against indomethacin-induced gastric ulcer in male albino rat were evaluated using hydromethanolic extract. The active ingredients present in the *A. triplinervis* shows gastroprotective properties that prevent the indomethacin-induced gastric ulcer.[35]

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CONFLICT OF INTEREST

Nil.

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