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NEGATIVE IMPACTS OF TRADITIONAL MEDICINE: ARISTOLOCHIC ACID MODEL

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ABSTRACT

The use of herbal preparations for curing various ailments have gained wide and increasing acceptance worldwide, because of the sound belief that they are 'natural' and hence 'safe'. However, no scientific validation and clinical experimentation have been documented for most of these traditional practices. This in turn has made the consumers prone to dangerous health problems caused by the unknown phytochemicals. This potential risk has been documented in this article by highlighting the dangerous effects of aristolochic acid. Presence of aristolochic acid (the nephrotoxic, carcinogenic and infertility agent) is reported for the first time from *Thottea siliquosa*, through TLC and HPLC procedures, an extensively used herbal remedy in southern Western Ghats.

KEYWORDS

Aristolochic acid, herbal (traditional) medicine, TLC, HPLC and *Thottea siliquosa*

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INTRODUCTION

Traditional medicine has been defined as "the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health, as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness[1]. In the past decade, there has been renewed attention and interest in the use of traditional medicine globally. In China, traditional medicine accounts for around 40 % of all healthcares delivered. In Chile, 71 % of the population and in Columbia 40 % of the population have used such

medicine. In India, 65 % of the rural population use traditional medicine to meet their primary healthcare needs. In developed countries, traditional, complementary and alternative medicines are becoming more popular[2].

Use of plants as a source of medicine has been inherited and is an important component of the healthcare system in India. There are about 45,000 plant species in India, with concentrated hot spots in the region of Eastern Himalayas, Western Ghats and Andaman & Nicobar Islands. The officially documented plants with medicinal potential are 3000, but traditional practitioners use more than 6000[3]. Most of the traditional medicinal practices in India are revolving around the tribal people inhabiting the forest wilderness. Some of those have been infiltrated outside and amalgamated with the known healthcare systems like Ayurveda. Many consumers use traditional medicine as self-care, because there is a wide misconception that 'natural means safe. The traditional healers as well as their consumers of traditional medicine may be unaware of the potential side-effects of these herbal drugs, and how and when they can be administered safely. Even though traditional medicine has long been used, there is little experimental evidence regarding its safety and efficacy. The evolution of traditional medicine has been influenced by cultural and historical conditions, making systematic evaluation difficult, since factors such as philosophy and theory which underline its use must be taken into account.

Thottea siliquosa, commonly called 'alpam', is an important constituent of several herbal formulations, and the root extracts of this plant are used traditionally for curing stomach ache. For tribal healers, this is an antidote to snake-bite[4]. But these practices are not having any scientific or experimental back up. *Thottea siliquosa* is coming under the family Aristolochiaceae, and most of the members of this family (*Aristolochia* spp., *Asarum* spp.) are notorious for having the carcinogenic chemical- aristolochic acid. But phytochemical screening for this dangerous phytochemical was not performed out in *T. siliquosa* till now, hence an attempt is made on here.

MATERIALS AND METHODS

The roots of *T. siliquosa* (Figure No.1) were collected from seven different localities of Western Ghats (Table No.1). The roots were sliced; air dried, powdered and kept in sealed polythene bags until used. Aristolochic acid extraction was performed according to Gaudreault et al.[5] Aristolochic acid standard was obtained from Sigma-Aldrich, and 1mg/ml working concentration was prepared in methanol. The TLC was done in Silica gel 60 F_{254} S aluminium plate (E-Merck, Germany). The mobile phase was acetonitrile/methanol/ water mixture solution (3:1:1). Detection was done under UV and photographed. Confirmation and quantification was done by HPLC (Shimadzu, Japan). The mobile phase was methanol: water (80:20) with 100mg/L SDS, pH was adjusted to 2 with phosphoric acid. The separation was done in a RP C_{18} column with a flow rate of 1.0 ml/ minute. The injection volume was 20 µl, and the separated components were detected by UV/VIS detector at 252 nm.

RESULTS

Thin layer chromatogram showed the presence of clear spots in line with the standard (Figure No.2). HPLC chromatograms (Figure No.3 & 4) further confirmed the presence of aristolochic acid in the root samples of *T. siliquosa*. Two aristlochic acid forms were finely resolved in the chromatogram. While quantifying, AA2 was identified as the major constituent in *T. siliquosa*, irrespective of the different accessions studied (Table No.2).

DISCUSSION

Aristolochic acids, a group of substituted 10-nitro-1-phenanthronic acids, have been found to occur in many species of the genus *Aristolochia,* as well as in other members of the family Aristolochiaceae[6]. Aristolochic acid is actually composed of aristolochic acid I (AA1) and aristolochic acid II (AA2), being the most abundant. There is clear evidence that these are genotoxic mutagens forming DNA adducts after metabolic activation through simple reduction of the nitro group[7]. The nephrotoxic and carcinogenic effects of aristolochic

acids in animals have been established[8-10]. Similar toxicities were observed in humans when a cluster of cases of chronic renal failure reported in Belgium was traced to the ingestion of a herbal preparation containing *Aristolochia fangchi*[11-16]. This syndrome initially referred to as the 'Chinese Herb Nephropathy' (CHN), then as 'Aristolochic Acid Nephropathy' (AAN), and more recently as Aristolochic Acid- Balkan Nephropathy'. Soon this was recognized as a global health problem[10, 14]. Aristolochic acids, when tested for carcinogenicity by oral administration in mice and rats, and by intraperitoneal injection in rabbits, induced forestomach carcinomas in mice and rats, and fibrotic changes in the kidney together with a low incidence of kidney tumours in rabbits. Subcutaneous injection of aristolochic acids into rats induced a low incidence of urothelial carcinomas in the kidney and malignant fibrohitiocytic sarcomas in the injection site[10]. Administration of aristolochic acid containing plant sources found to be effective in terminating pregnancy in mice, hamsters and rabbits[17-23]. In this sense, aristolochic acid is an antifertility agent also. The toxicological evidence of the risks associated with aristolochic acid is strong, and it is among the most potent 2 per cent of the carcinogens[24]. By considering these dangers, the Food and Drug Administration issued warnings and an important alert that herbal products are unsafe if they contain or are suspected to contain aristolochic acid[25]. Traditional medicinal practitioners in India and especially in Kerala are not aware about the real magnitude of aristolochic acid induced health problems. There is a widespread belief on the part of the general public that natural substances are inherently superior to synthetic substances with regard to efficacy and safety in matters related to human health, where *T. siliquosa* (alpam) is an excellent example. In the present study, both the TLC and HPLC analyses confirmed the presence of aristolochic acids (AA1 & AA2) from the root tissues of *T. siliquosa*. Variations are noticed in the quantity of AA1 and AA2 among different accessions collected from different localities, but still they are present in significantly higher dose. Since it is a plant derived secondary metabolite, the difference in quantity in different accessions may be attributed by the difference in climatic and edaphic factors. Theses roots are being continuously used in tracer quantities in almost all the ayurvedic preparations. So whenever an individual depend on the ayurvedic preparations consisting of the 'alpam' roots, the person may unknowingly ingests the hazardous aristolochic acids into their body. Since most of the ayurvedic treatments and formulations are meant for long durations, the patients will have to administer these hazardous chemicals (even in tracer quantities) for considerable period of time (sometimes for years), and that in turn may induce genetic damage. More elaborate and specific clinical trials are needed for explaining that.

When analyzing the tribal and traditional systems, they are deliberately using the root extracts of this plant for curing snake-bite induced lethalities, diarrhoea, stomach pain etc. This is not having any documentary evidences, but a specialized culture and related practices made them to depend on these cheap, easily and naturally available therapies. From our field experiences, it has been noted that the traditional and tribal healers are giving ample quantities of *T. siliquosa* root extracts to patients suffering from various illness. The results are astonishingly positive and quick, and this may be the reason why they wholeheartedly welcome these practices, in spite of the unknown dangers that they cause. Regarding the therapies for snake-bite, there are reports describing the activity of aristolochic acid in inhibiting the enzymes PLA2 and NNHI present in the venom of Viper and Cobra respectively[26 & 27]. Aristolochic acid was found to abolish the activity of NNHI[27]. According to Neetzel et al.[26], aristolochic acid is capable of inhibiting the enzymatic activity of the neurotoxic complex Vipoxin and its PLA2 component. So altogether, the administration of aristolochic acid containing plant drugs in traditional system of medicine in the form remedy to snake-bite has some experimental support. But, when correlating with the hazardous after effects of the chemical, it's use without a proper clinical trial is not justifiable.

Table No.1: Details of *T. siliquosa* accessions collected

Accession Number	Collected from
1	Palode, Thiruvananthapuram
2	Braemore, Thiruvananthapuram
3	Kallar, Thiruvananthapuram
4	Thenmala, Kollam
5	Konni, Pathanamthitta
6	Nilakkal, Pathanamthitta
7	Kulathupuzha, Kollam

Table No.2: Aristolochic acid composition in *Thottea siliquosa*

Accession Number	Quantity (mg/g)		
	AA1	**AA2**	**Total AA**
1	1.61±0.03	3.76±0.03	5.50±0.08
2	0.25±0.01	2.29±0.03	2.52±0.02
3	1.27±0.05	3.13±0.03	4.40±0.07
4	0.30±0.01	2.75±0.05	3.05±0.06
5	0.25±0.02	2.54±0.03	2.80±0.04
6	0.81±0.02	2.46±0.03	3.22±0.06
7	0.88±0.01	2.77±0.06	3.68±0.01

Figure No.1: *Thottea siliquosa* – Habit

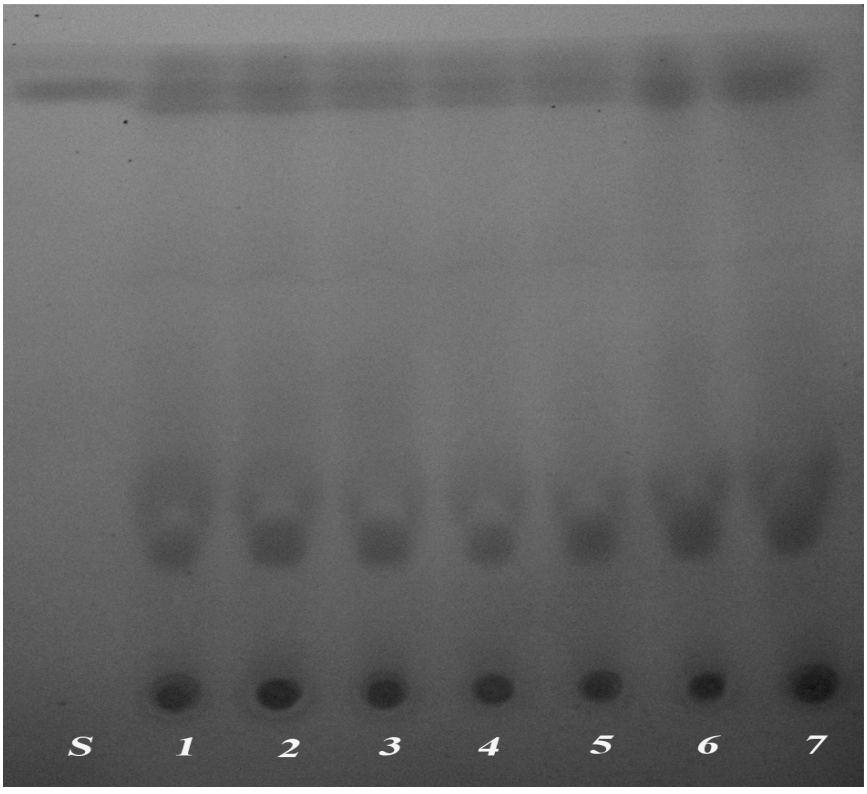

Figure No.2: Aristolochic acid profiling in the seven accessions of *T. siliquosa* by TLC. (S – Standard; 1 to 7 – accession numbers)

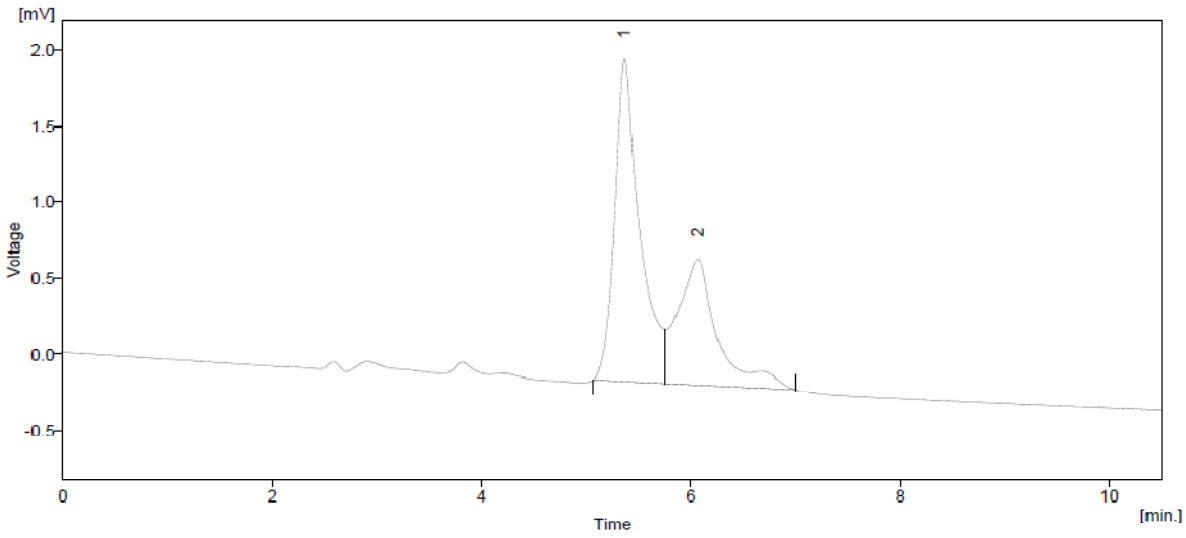

Figure No.3: HPLC profiling of aristolochic acid standard (1 – AA1; 2 – AA2)

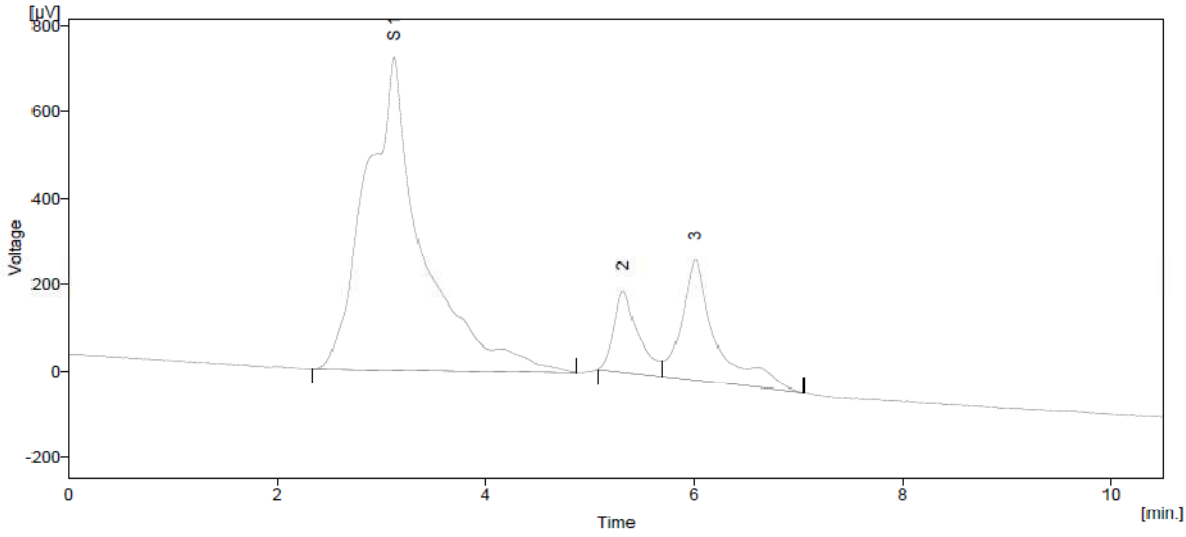

Figure No.4: HPLC profiling of root extracts of *T. siliquosa* (Accession Number 1) (2 – AA1; 3 – AA2)

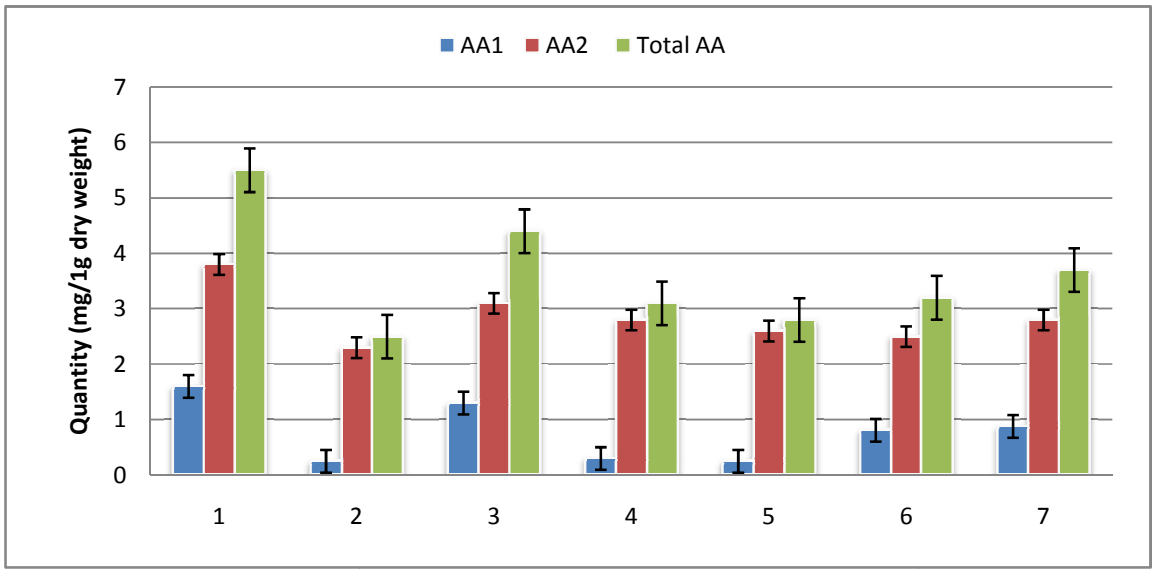

Figure No.5: Aristolochic acid composition in seven accessions of *T. siliquosa*

CONCLUSIONS

Though the traditional medicinal formulations and practices are cheap, natural, effective and "safe" (in a laymen concept), they have to be extensively investigated, both experimentally and clinically. So the traditional treatment with *T. siliquosa* roots and like plant materials should be scientifically validated and strict guidelines have to be formulated. There is an urgent need to protect the public from dangerous practices while permitting patient access to reasonably safe and effective therapies. The only way to achieve this is to submit all phytotherapy products to the same regulations as for any conventional drug. Whenever these rules and regulations become inadequate, awareness programmes should be administered by the authorities concerned, for the "safe" use of such natural herbal remedies.

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